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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C11D 3/386, C12N 9/54, A61K 7/48, 7/28, C11D 3/39

A1

(11) International Publication Number: WO 99/20726

(43) International Publication Date: 29 April 1999 (29.04.99)

(21) International Application Number: PCT/US98/22482

(22) International Filing Date: 23 October 1998 (23.10.98)

(30) Priority Data:

| | | |
|---|---|---|
| 08/956,323 | 23 October 1997 (23.10.97) | US |
| 08/956,564 | 23 October 1997 (23.10.97) | US |
| 08/956,324 | 23 October 1997 (23.10.97) | US |

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(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**

*With international search report.*

*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: BLEACHING COMPOSITIONS COMPRISING MULTIPLY-SUBSTITUTED PROTEASE VARIANTS

(57) Abstract

The present invention relates to bleaching compositions comprising a protease variant. One bleaching composition comprises a protease variant including a substitution of an amino acid residue with another naturally occurring amino acid residue at an amino acid residue position corresponding to position 103 of *Bacillus amyloliquefaciens* subtilisin in combination with a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274, and 275 of *Bacillus amyloliquefaciens* subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a substitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265, or 274 of *Bacillus amyloliquefaciens* subtilisin, a bleaching agent; and one or more cleaning adjunct materials. Another bleaching composition comprises a protease variant including a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin; a bleaching agent, and one or more cleaning adjunct materials. Methods for using the bleaching compositions are also provided.

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# BLEACHING COMPOSITIONS COMPRISING MULTIPLY-SUBSTITUTED PROTEASE VARIANTS

## FIELD OF THE INVENTION

The present invention relates to bleaching compositions, especially laundry detergents, which comprise one or more protease enzymes which are multiply-substituted protease variants and a bleaching system with one or more bleaching agents, especially bleach activators, and methods of using such bleaching compositions.

## BACKGROUND OF THE INVENTION

Various types of enzymes have long been conventionally used in laundry detergents to assist in the removal of certain stains from fabrics. These stains are typically associated with lipid and protein soils. The enzymes, however, have proven less effective against other types of soils and stains.

U.S. Patent No. 5,677,272 to Ghosh et al., issued October 10, 1997, discloses bleaching compositions comprising: 1) a protease variant including substitutions of amino acid residues with other amino acid residues at positions corresponding to positions 76 in combination with one or more of the following positions 99, 101, 103, 104, 107, 123, 27, 105, 109, 126, 128, 135, 156, 166, 195, 197, 204, 206, 210, 216, 217, 218, 222, 260, 265 and/or 274 of *Bacillus amyloliquefaciens* subtilisin; 2) a bleaching agent; and 3) one or

more bleaching composition materials compatible with the protease variant and bleaching agent.

However, a need for more effective stain removal and/or dingy cleanup over the conventional bleaching compositions still exists.

By the present invention, it has been found that the combination of novel protease enzymes which are multipy-substituted protease variants and bleaching agents, especially bleach activators, provide enhanced and improved stain removal and/or dingy cleanup benefits over conventional bleaching compositions.

Accordingly, it is an object of the present invention to provide bleaching compositions, especially laundry detergent compositions, having improved stain and/or soil removal and/or dingy cleanup benefits and/or fabric cleaning benefits and /or bleaching properties.

These and other objects of the present invention will be apparent from the detailed description hereinafter.

## SUMMARY OF THE INVENTION

The present invention meets the aforementioned needs in that it has been surprisingly discovered that the multiply-substituted protease variants of the present invention, when used in bleaching compositions provide improved and enhanced cleaning benefits, including, but not limited to, stain and/or soil removal and/or reduction and/or whiteness maintenance and/or dingy cleanup and/or spot and/or film removal and/or reduction, over conventional protease-containing bleaching compositions.

The multiply-substituted protease variants of the present invention are suitable for use in high and low density granular, heavy duty and light duty liquids, tablets, powders, gels, foams, sprays, paste, as well as synthetic detergent bar compositions, and other bleaching compositions.

In one aspect of the present invention a bleaching composition comprising:

(a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.0001% to about 10% by weight of the bleaching composition of a protease variant, wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at an amino acid residue position corresponding to position 103 of *Bacillus amyloliquefaciens* subtilisin in combination with a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147,

158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of *Bacillus amyloliquefaciens* subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a substitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of *Bacillus amyloliquefaciens* subtilisin;

(b) a bleaching agent which either is an organic peroxyacid or is a combination of a bleach activator and a peroxygen compound capable of yielding hydrogen peroxide that can react with the activator to form an organic peroxyacid in situ in a bleaching solution formed from the composition; and

(c) one or more cleaning adjunct materials.

In yet another aspect of the present invention, a fabric bleaching composition comprising:

(a) an effective amount, preferably from about 0.0001% to about 10% by weight of the fabric bleaching composition, of the protease variant described above;

(b) a bleaching agent which either is an organic peroxyacid or is a combination of a bleach activator and a peroxygen compound capable of yielding hydrogen peroxide that can react with the activator to form an organic peroxyacid in situ in a bleaching solution formed from the composition;

(c) at least about 5% by weight of the fabric bleaching composition of a surfactant; and

(d) at least about 5% by weight of the fabric bleaching composition of a builder, is provided.

In still another aspect of the present invention, a method for cleaning a fabric in need of cleaning comprising contacting the fabric with the fabric bleaching composition of the present invention is provided.

In still yet another aspect of the present invention, a dishwashing bleaching composition comprising:

(a) an effective amount, preferably from about 0.0001% to about 10% by weight of the dishwashing composition, of a protease variant described above;

(b) a bleaching agent which either is an organic peroxyacid or is a combination of a bleach activator and a peroxygen compound capable of yielding hydrogen peroxide that can

react with the activator to form an organic peroxyacid in situ in a bleaching solution formed from the composition; and

(c) from about 0.1% to about 10% by weight of a surfactant,

is provided.

In still yet another aspect of the present invention, a method for cleaning a dish in need of cleaning comprising contacting the dish with the dishwashing bleaching composition of the present invention is provided.

In still yet another aspect of the present invention, a personal cleansing composition comprising:

(a) an effective amount, preferably from about 0.001% to about 5% by weight of the personal cleansing composition, of a protease variant described above;

(b) a bleaching agent which either is an organic peroxyacid or is a combination of a bleach activator and a peroxygen compound capable of yielding hydrogen peroxide that can react with the activator to form an organic peroxyacid in situ in a bleaching solution formed from the composition; and

(c) from about 0.1% to about 95% by weight of the personal cleansing composition of a surfactant system; and

(d) optionally, from about 0.05% to about 50% by weight of the personal cleansing composition of an enzyme stabilizer,

is provided.

In still yet another aspect of the present invention, a method for personal cleansing of a part of the human or lower animal body in need of cleansing comprising contacting the part with the personal cleansing composition of the present invention is provided.

In still yet another aspect of the present invention, a bleaching composition comprising:

(a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.0001% to about 10% by weight of the bleaching composition of a protease variant, wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;

(b) a bleaching agent which either is an organic peroxyacid or is a combination of a bleach activator and a peroxygen compound capable of yielding hydrogen peroxide that can react with the activator to form an organic peroxyacid in situ in a bleaching solution formed from the composition; and

(c) one or more cleaning adjunct materials,

is provided.

In still yet another aspect of the present invention, a fabric bleaching composition comprising:

(a) an effective amount, preferably from about 0.0001% to about 10% by weight of the fabric bleaching composition, of a protease variant wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;

(b) a bleaching agent which either is an organic peroxyacid or is a combination of a bleach activator and a peroxygen compound capable of yielding hydrogen peroxide that can react with the activator to form an organic peroxyacid in situ in a bleaching solution formed from the composition;

(c) at least about 5% by weight of the fabric bleaching composition, of a surfactant; and

(d) at least about 5% by weight of the fabric bleaching composition, of a builder, is provided.

In still another aspect of the present invention, a method for cleaning a fabric in need of cleaning comprising contacting the fabric with the fabric bleaching composition of the present invention is provided.

In still yet another aspect of the present invention, a dishwashing bleaching composition comprising:

(a) an effective amount, preferably from about 0.0001% to about 10% by weight of the fabric bleaching composition, of a protease variant wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;

(b) a bleaching agent which either is an organic peroxyacid or is a combination of a bleach activator and a peroxygen compound capable of yielding hydrogen peroxide that can react with the activator to form an organic peroxyacid in situ in a bleaching solution formed from the composition; and

(c) from about 0.1% to about 10% by weight of the dishwashing composition, of a surfactant,

is provided.

In still yet another aspect of the present invention, a method for cleaning a dish in need of cleaning comprising contacting the dish with the dishwashing bleaching composition of the present invention is provided.

In still yet another aspect of the present invention, a personal cleansing composition comprising:

(a) an effective amount, preferably from about 0.001% to about 5% by weight of the personal cleansing composition, of a protease variant wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;

(b) a bleaching agent which either is an organic peroxyacid or is a combination of a bleach activator and a peroxygen compound capable of yielding hydrogen peroxide that can react with the activator to form an organic peroxyacid in situ in a bleaching solution formed from the composition; and

(c) from about 0.1% to about 95% by weight of the personal cleansing composition, of a surfactant system; and

(d) optionally, from about 0.05% to about 50% by weight of the personal cleansing composition, of an enzyme stabilizer.

is provided.

In still yet another aspect of the present invention, a method for personal cleansing of a part of the human or lower animal body in need of cleansing comprising contacting the part with the personal cleansing composition of the present invention is provided.

Accordingly, it is an object of the present invention to provide bleaching compositions having a protease variant capable of providing improved and enhanced cleaning of fabrics, dishware, tableware, kitchenware, cookware and other hard surface substrates. It is a further object of the present invention to provide methods for fabric, dishware, tableware, kitchenware, cookware and other hard surface substrate cleansing via the use of the protease variant-containing bleaching compositions of the present invention.

These and other objects, features and advantages will be clear from the following detailed description, examples and appended claims.

All percentages, ratios and proportions herein are on a weight basis unless otherwise indicated. All documents cited herein are hereby incorporated by reference.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1 A-C depict the DNA and amino acid sequence for *Bacillus amyloliquefaciens* subtilisin and a partial restriction map of this gene.

Fig. 2 depicts the conserved amino acid residues among subtilisins from *Bacillus amyloliquefaciens* (BPN)' and *Bacillus lentus* (wild-type).

Figs. 3A and 3B depict the amino acid sequence of four subtilisins. The top line represents the amino acid sequence of subtilisin from *Bacillus amyloliquefaciens* subtilisin (also sometimes referred to as subtilisin BPN'). The second line depicts the amino acid sequence of subtilisin from *Bacillus subtilis*. The third line depicts the amino acid

sequence of subtilisin from *B. lichenifformis*. The fourth line depicts the amino acid sequence of subtilisin from *Bacillus lentus* (also referred to as subtilisin 309 in PCT WO89/06276). The symbol * denotes the absence of specific amino acid residues as compared to subtilisin BPN'.

## DETAILED DESCRIPTION OF THE INVENTION

The bleaching compositions employed in the present invention provide improved and enhanced cleaning of fabrics, dishware, kitchenware, tableware, and other hard surfaces as more fully described herein by removing and/or reducing soils and/or stains from the fabrics and other hard surfaces, and by removing and/or reducing spotting and/or filming from the dishware and other hard surfaces.

The bleaching systems in combination with the protease enzymes of the present invention are particularly efficient and effective at removing most types of soils from fabrics, including protein and lipid soils, dingy soils, and heavy soil loads, especially nucleophilic and body soils.

The protease enzymes, bleaching agents (including peroxyacids and bleaching systems) and cleaning adjunct materials useful herein, including preferred levels, are described in detail hereinafter.

I. Proteases - Proteases are carbonyl hydrolases which generally act to cleave peptide bonds of proteins or peptides. As used herein, "protease" means a naturally occurring protease or recombinant protease. Naturally-occurring proteases include α-aminoacylpeptide hydrolase, peptidylamino acid hydrolase, acylamino hydrolase, serine carboxypeptidase, metallocarboxypeptidase, thiol proteinase, carboxylproteinase and metalloproteinase. Serine, metallo, thiol and acid protease are included, as well as endo and exo-proteases.

The present invention includes protease enzymes which are non-naturally occurring carbonyl hydrolase variants (protease variants) having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Specifically, such protease variants have an amino acid sequence not found in nature, which is derived by replacement of a plurality of amino acid residues of a precursor protease with different amino acids. The precursor protease may be a naturally-occurring protease or recombinant protease. As stated earlier, the protease variants are designed to have trypsin-like specificity and preferably also be bleach stable.

The protease variants useful herein encompass the substitution of any of the nineteen naturally occurring L-amino acids at the designated amino acid residue positions. Such substitutions can be made in any precursor subtilisin (procaryotic, eucaryotic,

mammalian, etc.). Throughout this application reference is made to various amino acids by way of common one- and three-letter codes. Such codes are identified in Dale, M.W. (1989), Molecular Genetics of Bacteria, John Wiley & Sons, Ltd., Appendix B.

The protease variants useful herein are preferably derived from a *Bacillus* subtilisin. More preferably, the protease variants are derived from *Bacillus lentus* subtilisin and/or subtilisin 309.

Carbonyl Hydrolases - Carbonyl hydrolases are protease enzymes which hydrolyze compounds containing

bonds in which X is oxygen or nitrogen. They include naturally-occurring carbonyl hydrolases and recombinant carbonyl hydrolases. Naturally-occurring carbonyl hydrolases principally include hydrolases, e.g., peptide hydrolases such as subtilisins or metalloproteases. Peptide hydrolases include α-aminoacylpeptide hydrolase, peptidylamino acid hydrolase, acylamino hydrolase, serine carboxypeptidase, metallocarboxypeptidase, thiol proteinase, carboxylproteinase and metalloproteinase. Serine, metallo, thiol and acid protease's are included, as well as endo and exo-proteases.

Subtilisins - Subtilisins are bacterial or fungal proteases which generally act to cleave peptide bonds of proteins or peptides. As used herein, "subtilisin" means a naturally-occurring subtilisin or a recombinant subtilisin. A series of naturally-occurring subtilisins is known to be produced and often secreted by various microbial species. Amino acid sequences of the members of this series are not entirely homologous. However, the subtilisins in this series exhibit the same or similar type of proteolytic activity. This class of serine proteases share a common amino acid sequence defining a catalytic triad which distinguishes them from the chymotrypsin related class of serine proteases. The subtilisins and chymotrypsin related serine proteases both have a catalytic triad comprising aspartate, histidine and serine. In the subtilisin related proteases the relative order of these amino acids, reading from amino to carboxy terminus, is aspartate-histidine-serine. In the chymotrypsin related proteases, the relative order, however, is histidine-aspartate-serine. Thus, subtilisin herein refers to a serine protease having the catalytic triad of subtilisin related proteases. Examples include, but are not limited to, the subtilisins identified in Fig. 3 herein. Generally, and for purposes of the present invention, numbering of the amino acids in proteases corresponds to the numbers assigned to the mature *Bacillus amyloliquefaciens* subtilisin sequence presented in Fig. 1.

Protease Variants - A "protease variant" has an amino acid sequence which is derived from the amino acid sequence of a "precursor protease." The precursor proteases

include naturally-occurring proteases and recombinant proteases. The amino acid sequence of the protease variant is "derived" from the precursor protease amino acid sequence by substitution, deletion or insertion of one or more amino acids of the precursor amino acid sequence. Such modification is of the "precursor DNA sequence" which encodes the amino acid sequence of the precursor protease rather than manipulation of the precursor protease enzyme *per se*. Suitable methods for such manipulation of the precursor DNA sequence include methods disclosed herein, as well as methods know to those skilled in the art (see, for example, EP 0 328 299, WO 89/06279 and the U.S. patents and applications already referenced herein).

In a preferred embodiment, the protease variants which are protease enzymes useful in the present invention bleaching compositions comprise protease variants including a substitution of an amino acid residue with another naturally occurring amino acid residue at an amino acid residue position corresponding to position 103 of *Bacillus amyloliquefaciens* subtilisin in combination with a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of *Bacillus amyloliquefaciens* subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a substitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of *Bacillus amyloliquefaciens* subtilisin; and one or more cleaning adjunct materials.

While any combination of the above listed amino acid substitutions may be employed, the preferred protease variant enzymes useful for the present invention comprise the substitution, deletion or insertion of amino acid residues in the following combinations:

(1) a protease variant including substitutions of the amino acid residues at position 103 and at one or more of the following positions 236 and 245;

(2) a protease variant including substitutions of the amino acid residues at positions 103 and 236 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101,

102, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 211, 212, 213, 215, 217, 230, 232, 248, 252, 257, 260, 270 and 275;

(3) a protease variant including substitutions of the amino acid residues at positions 103 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 104, 109, 130, 131, 159, 170, 183, 185, 205, 209, 210, 211, 212, 213, 215, 217, 222, 230, 232, 248, 252, 257, 260, 261, 270 and 275; and

(4) a protease variant including substitutions of the amino acid residues at positions 103, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 211, 212, 213, 215, 217, 230, 232, 243, 248, 252, 257, 260, 270 and 275.

A more preferred protease variant useful in the cleaning compositions of the present invention include a substitution set (one substitution set per row in the following Table I) selected from the group consisting of:

Table I

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 76 | 98 | 103 | 104 | | | | | | | | | |
| 76 | 78 | 103 | 104 | | | | | | | | | |
| 76 | 103 | 104 | 107 | | | | | | | | | |
| 4 | 76 | 103 | 104 | | | | | | | | | |
| 76 | 103 | 104 | 246 | | | | | | | | | |
| 76 | 77 | 103 | 104 | | | | | | | | | |
| 76 | 103 | 104 | 183 | 218 | | | | | | | | |
| 16 | 76 | 103 | 104 | 248 | | | | | | | | |
| 1 | 76 | 103 | 104 | | | | | | | | | |
| 76 | 103 | 104 | 261 | | | | | | | | | |
| 76 | 103 | 104 | 160 | | | | | | | | | |
| 76 | 103 | 104 | 216 | | | | | | | | | |
| 17 | 76 | 103 | 104 | | | | | | | | | |
| 37 | 76 | 103 | 104 | | | | | | | | | |
| 76 | 77 | 103 | 104 | 174 | | | | | | | | |
| 38 | 76 | 103 | 104 | | | | | | | | | |
| 38 | 76 | 103 | 104 | 237 | | | | | | | | |
| 8 | 76 | 103 | 104 | | | | | | | | | |

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| 76 | 103 | 104 | 183 | | | | | | | | | |
| 19 | 76 | 103 | 104 | | | | | | | | | |
| 13 | 76 | 103 | 104 | | | | | | | | | |
| 19 | 76 | 103 | 104 | | | | | | | | | |
| 76 | 103 | 104 | 184 | | | | | | | | | |
| 76 | 103 | 104 | 252 | | | | | | | | | |
| 76 | 103 | 104 | 259 | | | | | | | | | |
| 76 | 103 | 104 | 251 | | | | | | | | | |
| 76 | 86 | 103 | 104 | | | | | | | | | |
| 72 | 76 | 103 | 104 | 185 | | | | | | | | |
| 76 | 103 | 104 | 237 | 274 | | | | | | | | |
| 76 | 103 | 104 | 160 | | | | | | | | | |
| 76 | 103 | 104 | 228 | | | | | | | | | |
| 55 | 76 | 103 | 104 | 240 | | | | | | | | |
| 76 | 103 | 104 | 254 | | | | | | | | | |
| 76 | 103 | 104 | 204 | | | | | | | | | |
| 76 | 103 | 104 | 204 | | | | | | | | | |
| 43 | 76 | 103 | 104 | | | | | | | | | |
| 76 | 103 | 104 | 159 | | | | | | | | | |
| 10 | 76 | 103 | 104 | 177 | | | | | | | | |
| 58 | 76 | 103 | 104 | | | | | | | | | |
| 76 | 103 | 104 | 270 | | | | | | | | | |
| 76 | 103 | 104 | 185 | | | | | | | | | |
| 27 | 76 | 103 | 104 | | | | | | | | | |
| 76 | 103 | 104 | 262 | | | | | | | | | |
| 76 | 78 | 103 | 104 | | | | | | | | | |
| 24 | 76 | 103 | 104 | | | | | | | | | |
| 76 | 103 | 104 | 166 | 236 | 251 | | | | | | | |
| 17 | 76 | 103 | 104 | 237 | | | | | | | | |
| 76 | 103 | 104 | 130 | | | | | | | | | |
| 76 | 103 | 104 | 109 | | | | | | | | | |
| 76 | 99 | 103 | 104 | 204 | | | | | | | | |

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| 76 | 103 | 104 | 181 | | | | | | | | | |
| 12 | 76 | 103 | 104 | | | | | | | | | |
| 76 | 103 | 104 | 212 | 271 | | | | | | | | |
| 76 | 103 | 104 | 252 | 261 | | | | | | | | |
| 76 | 103 | 104 | 242 | | | | | | | | | |
| 76 | 103 | 104 | 271 | | | | | | | | | |
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| 76 | 103 | 104 | 271 | | | | | | | | | |
| 61 | 76 | 103 | 104 | | | | | | | | | |
| 38 | 76 | 103 | 104 | 182 | 263 | | | | | | | |
| 76 | 103 | 104 | 182 | 272 | | | | | | | | |
| 76 | 103 | 104 | 109 | 246 | | | | | | | | |
| 76 | 87 | 103 | 104 | 206 | 249 | 265 | | | | | | |
| 76 | 103 | 104 | 137 | 238 | 271 | | | | | | | |
| 103 | 104 | 228 | | | | | | | | | | |
| 76 | 103 | 104 | 182 | 198 | | | | | | | | |
| 21 | 76 | 103 | 104 | 182 | | | | | | | | |
| 76 | 103 | 104 | 119 | 137 | | | | | | | | |
| 76 | 103 | 104 | 137 | 248 | | | | | | | | |
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| 76 | 103 | 104 | 212 | 258 | | | | | | | | |
| 58 | 76 | 103 | 104 | 271 | | | | | | | | |
| 76 | 103 | 104 | 206 | 261 | | | | | | | | |
| 4 | 76 | 103 | 104 | 206 | | | | | | | | |
| 76 | 77 | 103 | 104 | 206 | | | | | | | | |
| 76 | 103 | 104 | 158 | | | | | | | | | |
| 76 | 103 | 104 | 206 | | | | | | | | | |
| 4 | 76 | 103 | 104 | 159 | 217 | 251 | | | | | | |
| 4 | 76 | 103 | 104 | 159 | 217 | 252 | | | | | | |

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| 76 | 77 | 103 | 104 | 133 | 185 | 251 | | | | | | |
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| 4 | 76 | 103 | 104 | 188 | | | | | | | | |
| 4 | 76 | 103 | 104 | 158 | | | | | | | | |
| 76 | 77 | 103 | 104 | 185 | | | | | | | | |
| 76 | 103 | 104 | 206 | 251 | | | | | | | | |
| 48 | 76 | 103 | 104 | 111 | 159 | | | | | | | |
| 68 | 76 | 103 | 104 | 159 | 236 | | | | | | | |
| 42 | 76 | 103 | 104 | 159 | | | | | | | | |
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| 76 | 103 | 104 | 146 | 159 | | | | | | | | |
| 76 | 103 | 104 | 159 | 238 | | | | | | | | |
| 76 | 103 | 104 | 159 | 224 | | | | | | | | |
| 76 | 103 | 104 | 212 | 268 | 271 | | | | | | | |
| 76 | 89 | 103 | 104 | | | | | | | | | |
| 76 | 87 | 103 | 104 | 212 | 271 | | | | | | | |
| 76 | 103 | 104 | 212 | 245 | 271 | | | | | | | |
| 76 | 103 | 104 | 134 | 141 | 212 | 271 | | | | | | |
| 76 | 103 | 104 | 212 | 236 | 243 | 271 | | | | | | |
| 76 | 103 | 104 | 109 | 245 | | | | | | | | |
| 76 | 103 | 104 | 109 | 210 | | | | | | | | |
| 20 | 62 | 76 | 103 | 104 | | | | | | | | |
| 68 | 76 | 103 | 104 | 236 | | | | | | | | |
| 68 | 76 | 103 | 104 | 159 | 236 | 271 | | | | | | |
| 68 | 76 | 103 | 104 | 159 | 236 | 245 | | | | | | |
| 68 | 76 | 103 | 104 | 159 | 217 | 236 | 271 | | | | | |
| 17 | 68 | 76 | 103 | 104 | | | | | | | | |
| 68 | 76 | 103 | 104 | | | | | | | | | |
| 68 | 76 | 103 | 104 | 159 | 236 | | | | | | | |
| 68 | 75 | 76 | 103 | 104 | 159 | 236 | | | | | | |
| 68 | 76 | 76 | 103 | 114 | 121 | 159 | 236 | 245 | | | | |

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| 12 | 68 | 76 | 103 | 104 | 159 | 236 | | | | | | |
| 68 | 76 | 103 | 104 | 159 | 209 | 236 | 253 | | | | | |
| 68 | 76 | 103 | 104 | 117 | 159 | 184 | 236 | | | | | |
| 68 | 76 | 103 | 104 | 159 | 236 | 243 | | | | | | |
| 68 | 76 | 103 | 104 | 159 | 236 | 245 | | | | | | |
| 68 | 76 | 103 | 104 | 142 | 159 | | | | | | | |
| 68 | 76 | 103 | 104 | 123 | 159 | 236 | 249 | | | | | |
| 68 | 76 | 103 | 104 | 159 | 236 | 249 | | | | | | |
| 76 | 103 | 104 | 222 | 245 | | | | | | | | |
| 12 | 76 | 103 | 104 | 222 | 249 | | | | | | | |
| 76 | 103 | 104 | 173 | 222 | | | | | | | | |
| 76 | 103 | 104 | 222 | 263 | | | | | | | | |
| 21 | 76 | 103 | 104 | 222 | 237 | 263 | | | | | | |
| 76 | 103 | 104 | 109 | 222 | | | | | | | | |
| 76 | 103 | 104 | 109 | 222 | 271 | | | | | | | |
| 61 | 76 | 103 | 104 | 222 | | | | | | | | |
| 76 | 103 | 104 | 137 | 222 | | | | | | | | |
| 76 | 103 | 104 | 109 | 222 | 248 | | | | | | | |
| 76 | 103 | 104 | 222 | 249 | | | | | | | | |
| 68 | 76 | 103 | 104 | 159 | 236 | 245 | 261 | | | | | |
| 68 | 76 | 103 | 104 | 141 | 159 | 236 | 245 | 255 | | | | |
| 68 | 76 | 103 | 104 | 159 | 236 | 245 | 247 | | | | | |
| 68 | 76 | 103 | 104 | 159 | 174 | 204 | 236 | 245 | | | | |
| 68 | 76 | 103 | 104 | 159 | 204 | 236 | 245 | | | | | |
| 68 | 76 | 103 | 104 | 133 | 159 | 218 | 236 | 245 | | | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | | | |
| 68 | 76 | 103 | 104 | 159 | 194 | 203 | 236 | 245 | | | | |
| 12 | 76 | 103 | 104 | 222 | 245 | | | | | | | |
| 76 | 103 | 104 | 232 | 245 | | | | | | | | |
| 24 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 252 | | | | | |
| 68 | 76 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 260 | | | |

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| 12 | 76 | 103 | 104 | 222 | 244 | 245 | | | | | | |
| 12 | 76 | 103 | 222 | 210 | 245 | | | | | | | |
| 12 | 76 | 103 | 104 | 130 | 222 | 245 | | | | | | |
| 22 | 68 | 76 | 103 | 104 | | | | | | | | |
| 68 | 76 | 103 | 104 | 184 | | | | | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | | | | | | |
| 68 | 103 | 104 | 140 | 159 | 232 | 236 | 245 | 252 | | | | |
| 43 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 252 | | | | |
| 43 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | | | | | |
| 43 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 252 | | | | |
| 68 | 87 | 103 | 104 | 159 | 232 | 236 | 245 | 252 | 275 | | | |
| 12 | 76 | 103 | 104 | 130 | 222 | 245 | 248 | 262 | | | | |
| 12 | 76 | 103 | 104 | 130 | 215 | 222 | 245 | | | | | |
| 12 | 76 | 103 | 104 | 130 | 222 | 227 | 245 | 262 | | | | |
| 12 | 76 | 103 | 104 | 130 | 222 | 245 | 261 | | | | | |
| 76 | 103 | 104 | 130 | 222 | 245 | | | | | | | |
| 12 | 76 | 103 | 104 | 130 | 218 | 222 | 245 | 262 | 269 | | | |
| 12 | 57 | 76 | 103 | 104 | 130 | 222 | 245 | 251 | | | | |
| 12 | 76 | 103 | 104 | 130 | 170 | 185 | 222 | 243 | 245 | | | |
| 12 | 76 | 103 | 104 | 130 | 222 | 245 | 268 | | | | | |
| 12 | 76 | 103 | 104 | 130 | 222 | 210 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 257 | | | | | |
| 68 | 103 | 104 | 116 | 159 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | | | | | |
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| 68 | 103 | 104 | 159 | 203 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 237 | 245 | | | | | |
| 68 | 76 | 79 | 103 | 104 | 159 | 232 | 236 | 245 | | | | |
| 68 | 103 | 104 | 159 | 183 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 174 | 206 | 232 | 236 | 245 | | | | |
| 68 | 103 | 104 | 159 | 188 | 232 | 236 | 245 | | | | | |

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| 68 | 103 | 104 | 159 | 230 | 232 | 236 | 245 | | | | | |
| 68 | 98 | 103 | 104 | 159 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 215 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | | | | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | | | |
| 68 | 76 | 103 | 104 | 159 | 210 | 232 | 236 | 245 | | | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 257 | | | | |
| 76 | 103 | 104 | 232 | 236 | 245 | 257 | | | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 257 | 275 | | | | |
| 76 | 103 | 104 | 257 | 275 | | | | | | | | |
| 68 | 103 | 104 | 159 | 224 | 232 | 236 | 245 | 257 | | | | |
| 76 | 103 | 104 | 159 | 232 | 236 | 245 | 257 | | | | | |
| 68 | 76 | 103 | 104 | 159 | 209 | 232 | 236 | 245 | | | | |
| 68 | 76 | 103 | 104 | 159 | 211 | 232 | 236 | 245 | | | | |
| 12 | 68 | 76 | 103 | 104 | 159 | 214 | 232 | 236 | 245 | | | |
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| 12 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | | |
| 20 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 259 | | | |
| 68 | 87 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 260 | | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 261 | | | | |
| 76 | 103 | 104 | 232 | 236 | 242 | 245 | | | | | | |
| 68 | 76 | 103 | 104 | 159 | 210 | 232 | 236 | 245 | | | | |
| 12 | 48 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | |
| 76 | 103 | 104 | 232 | 236 | 245 | | | | | | | |
| 76 | 103 | 104 | 159 | 192 | 232 | 236 | 245 | | | | | |
| 76 | 103 | 104 | 147 | 159 | 232 | 236 | 245 | 248 | 251 | | | |
| 12 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 272 | | | |
| 68 | 76 | 103 | 104 | 159 | 183 | 206 | 232 | 236 | 245 | | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 256 | | | | |
| 68 | 76 | 103 | 104 | 159 | 206 | 232 | 236 | 245 | | | | |
| 27 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | | |
| 68 | 76 | 103 | 104 | 116 | 159 | 170 | 185 | 232 | 236 | 245 | | |

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| 61 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 43 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
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| 68 | 103 | 104 | 159 | 209 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 109 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 20 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
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| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
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| 8 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 269 | | |
| 68 | 103 | 104 | 116 | 159 | 232 | 236 | 245 | 248 | 252 | 260 | | |
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| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 261 | | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
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| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
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| 68 | 103 | 104 | 159 | 218 | 232 | 236 | 245 | 248 | 252 | | | |
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| 103 | 104 | 159 | 230 | 236 | 245 | | | | | | | |
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| 68 | 103 | 104 | 159 | 174 | 232 | 236 | 245 | 257 | | | | |
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| 101 | 103 | 104 | 159 | 185 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 103 | 104 | 159 | 206 | 232 | 236 | 245 | 248 | 252 | | | |

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| 62 | 103 | 104 | 109 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 62 | 103 | 104 | 159 | 212 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 62 | 101 | 103 | 104 | 159 | 212 | 213 | 232 | 236 | 245 | 248 | 252 | |
| 103 | 104 | 159 | 232 | 245 | 248 | 252 | | | | | | |
| 103 | 104 | 159 | 230 | 245 | | | | | | | | |
| 62 | 103 | 104 | 130 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 101 | 103 | 104 | 130 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 103 | 104 | 128 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 62 | 101 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 62 | 103 | 104 | 128 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 62 | 103 | 104 | 128 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 101 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 260 | | | |
| 101 | 103 | 104 | 131 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 98 | 101 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 99 | 101 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 103 | 104 | 159 | 212 | 232 | 236 | 245 | 248 | 252 | | | |
| 76 | 103 | 104 | 167 | 170 | 194 | | | | | | | |
| 101 | 103 | 104 | 159 | 209 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 103 | 104 | 159 | 210 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 103 | 104 | 159 | 205 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 103 | 104 | 159 | 230 | 236 | 245 | | | | | | |
| 101 | 103 | 104 | 159 | 194 | 232 | 236 | 245 | 248 | 252 | | | |
| 76 | 101 | 103 | 104 | 159 | 194 | 232 | 236 | 245 | 248 | 252 | | |
| 101 | 103 | 104 | 159 | 230 | 232 | 236 | 245 | 248 | 252 | | | |
| 62 | 103 | 104 | 159 | 185 | 206 | 213 | 232 | 236 | 245 | 248 | 252 | 271 |

An even more preferred protease variant useful in the cleaning compositions of the present invention include a substitution set (one substitution set per row in the following Table II) selected from the group consisting of:

Table II

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N76D | A98E | S103A | V104I | | | | | | | | | |
| N76D | S78T | S103A | V104I | | | | | | | | | |
| N76D | S103A | V104I | I107V | | | | | | | | | |
| V4E | N76D | S103A | V104I | | | | | | | | | |
| N76D | S103A | V104I | I246V | | | | | | | | | |
| N76D | N77D | S103A | V104I | | | | | | | | | |
| N76D | S103A | V104I | N183D | N218I | | | | | | | | |
| A16T | N76D | S103A | V104I | N248D | | | | | | | | |
| A1E | N76D | S103A | V104I | | | | | | | | | |
| N76D | S103A | V104I | N261D | | | | | | | | | |
| N76D | S103A | V104I | S160T | | | | | | | | | |
| N76D | S103A | V104I | S216C | | | | | | | | | |
| H17Q | N76D | S103A | V104I | | | | | | | | | |
| S37T | N76D | S103A | V104I | | | | | | | | | |
| N76D | N77D | S103A | V104I | A174V | | | | | | | | |
| T38S | N76D | S103A | V104I | | | | | | | | | |
| T38S | N76D | S103A | V104I | K237Q | | | | | | | | |
| I8V | N76D | S103A | V104I | | | | | | | | | |
| N76D | S103A | V104I | N183D | | | | | | | | | |
| R19L | N76D | S103A | V104I | | | | | | | | | |
| A13V | N76D | S103A | V104I | | | | | | | | | |
| R19C | N76D | S103A | V104I | | | | | | | | | |
| N76D | S103A | V104I | N184D | | | | | | | | | |
| N76D | S103A | V104I | N252D | | | | | | | | | |
| N76D | S103A | V104I | S259C | | | | | | | | | |
| N76D | S103A | V104I | K251T | | | | | | | | | |
| N76D | P86S | S103A | V104I | | | | | | | | | |
| I72V | N76D | S103A | V104I | N185D | | | | | | | | |
| N76D | S103A | V104I | K237E | T274A | | | | | | | | |
| N76D | S103A | V104I | S160L | | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N76D | S103A | V104I | A228V | | | | | | | | | |
| P55S | N76D | S103A | V104I | S240T | | | | | | | | |
| N76D | S103A | V104I | A254T | | | | | | | | | |
| N76D | S103A | I104N | N204T | | | | | | | | | |
| N76D | S103A | V104I | N204D | | | | | | | | | |
| N43S | N76D | S103A | V104I | | | | | | | | | |
| N76D | S103A | V104I | G159D | | | | | | | | | |
| R10H | N76D | S103A | V104I | V177A | | | | | | | | |
| T58S | N76D | S103A | V104I | | | | | | | | | |
| N76D | S103A | V104I | A270V | | | | | | | | | |
| N76D | S103A | V104I | N185D | | | | | | | | | |
| K27N | N76D | S103A | V104I | | | | | | | | | |
| N76D | S103A | V104I | L262M | | | | | | | | | |
| N76D | S78P | S103A | V104I | | | | | | | | | |
| S24P | N76D | S103A | V104I | | | | | | | | | |
| N76D | S103A | V104I | S166G | Q236R | K251R | | | | | | | |
| H17L | N76D | S103A | V104I | K237E | | | | | | | | |
| N76D | S103A | V104I | S130L | | | | | | | | | |
| N76D | S103A | V104I | Q109R | | | | | | | | | |
| N76D | S99R | S103A | V104I | N204T | | | | | | | | |
| N76D | S103A | V104I | D181N | | | | | | | | | |
| Q12R | N76D | S103A | V104I | | | | | | | | | |
| N76D | S103A | V104I | S212P | E271V | | | | | | | | |
| N76D | S103A | V104I | N252K | N261Y | | | | | | | | |
| N76D | S103A | V104I | S242T | | | | | | | | | |
| N76D | S103A | V104I | E271Q | | | | | | | | | |
| Q12R | N76D | S103A | V104I | S242T | | | | | | | | |
| N43S | N76D | S103A | V104I | N116K | N183I | | | | | | | |
| N76D | S103A | V104I | G258R | | | | | | | | | |
| N76D | S103A | V104I | E271G | | | | | | | | | |
| G61R | N76D | S103A | V104I | | | | | | | | | |
| T38S | N76D | S103A | V104I | Q182R | Y263H | | | | | | | |
| N76D | S103A | V104I | Q182R | A272S | | | | | | | | |
| N76D | S103A | V104I | Q109R | I246V | | | | | | | | |
| N76D | S87G | S103A | V104I | Q206R | H249Q | S265G | | | | | | |
| N76D | S103A | V104I | Q137R | N238Y | E271V | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S103A | V104I | A228T | | | | | | | | | | |
| N76D | S103A | V104I | Q182R | I198V | | | | | | | | |
| L21M | N76D | S103A | V104I | Q182R | | | | | | | | |
| N76D | S103A | V104I | M119I | Q137R | | | | | | | | |
| N76D | S103A | V104I | Q137R | N248S | | | | | | | | |
| A13T | N76D | S103A | V104I | Q206R | | | | | | | | |
| N76D | S103A | V104I | Q206R | | | | | | | | | |
| N76D | S103A | V104I | S212P | G258R | | | | | | | | |
| T58S | N76D | S103A | V104I | E271G | | | | | | | | |
| N76D | S103A | V104I | Q206E | N261D | | | | | | | | |
| V4E | N76D | S103A | V104I | Q206E | | | | | | | | |
| N76D | N77D | S103A | V104I | Q206E | | | | | | | | |
| N76D | S103A | V104I | A158E | | | | | | | | | |
| N76D | S103A | V104I | Q206E | | | | | | | | | |
| V4E | N76D | S103A | V104I | G159D | L217E | K251Q | | | | | | |
| V4E | N76D | S103A | V104I | G159D | L217E | N252D | | | | | | |
| N76D | N77D | S103A | V104I | A133T | N185D | K251T | | | | | | |
| N76D | S103A | V104I | G159D | Q206E | V244A | | | | | | | |
| V4E | N76D | S103A | V104I | S188E | | | | | | | | |
| V4E | N76D | S103A | V104I | A158E | | | | | | | | |
| N76D | N77D | S103A | V104I | N185D | | | | | | | | |
| N76D | S103A | V104I | Q206E | K251T | | | | | | | | |
| A48T | N76D | S103A | V104I | L111M | G159D | | | | | | | |
| V68A | N76D | S103A | V104I | G159D | Q236H | | | | | | | |
| L42V | N76D | S103A | V104I | G159D | | | | | | | | |
| Q12H | N62H | N76D | S103A | V104I | G159D | | | | | | | |
| L42I | N76D | S103A | V104I | G159D | | | | | | | | |
| N76D | S103A | V104I | G146S | G159D | | | | | | | | |
| N76D | S103A | V104I | G159D | N238S | | | | | | | | |
| N76D | S103A | V104I | G159D | T224A | | | | | | | | |
| N76D | S103A | V104I | S212P | V268F | E271V | | | | | | | |
| N76D | E89A | S103A | V104I | | | | | | | | | |
| N76D | S87R | S103A | V104I | S212P | E271V | | | | | | | |
| N76D | S103A | V104I | S212P | Q245L | E271V | | | | | | | |
| N76D | S103A | V104I | T134S | S141N | S212P | E271V | | | | | | |
| N76D | S103A | V104I | S212P | Q236L | N243S | E271V | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N76D | S103A | V104I | Q109R | Q245R | | | | | | | | |
| N76D | S103A | V104I | Q109R | P210L | | | | | | | | |
| G20V | N62S | N76D | S103A | V104I | | | | | | | | |
| V68A | N76D | S103A | V104I | Q236H | | | | | | | | |
| V68A | N76D | S103A | V104I | G159D | Q236H | E271V | | | | | | |
| V68A | N76D | S103A | V104I | G159D | Q236H | Q245R | | | | | | |
| V68A | N76D | S103A | V104I | G159D | L217I | Q236H | E271V | | | | | |
| H17Q | V68A | N76D | S103A | V104I | | | | | | | | |
| V68A | N76D | S103A | V104I | | | | | | | | | |
| V68A | N76D | S103A | V104I | G159D | Q236R | | | | | | | |
| V68A | L75R | N76D | S103A | V104I | G159D | Q236H | | | | | | |
| V68A | N76D | N76D | S103A | A114V | V121I | G159D | Q236H | Q245R | | | | |
| Q12R | V68A | N76D | S103A | V104I | G159D | Q236H | | | | | | |
| V68A | N76D | S103A | V104I | G159D | Y209S | Q236H | T253K | | | | | |
| V68A | N76D | S103A | V104I | N117K | G159D | N184S | Q236H | | | | | |
| V68A | N76D | S103A | V104I | G159D | Q236H | N243I | | | | | | |
| V68A | N76D | S103A | V104I | G159D | Q236H | Q245L | | | | | | |
| V68A | N76D | S103A | V104I | A142V | G159D | | | | | | | |
| V68A | N76D | S103A | V104I | N123S | G159D | Q236H | H249Y | | | | | |
| V68A | N76D | S103A | V104I | G159D | Q236H | H249Q | | | | | | |
| N76D | S103A | V104I | M222S | Q245R | | | | | | | | |
| Q12R | N76D | S103A | V104I | M222S | H249R | | | | | | | |
| N76D | S103A | V104I | N173R | M222S | | | | | | | | |
| N76D | S103A | V104I | M222S | Y263F | | | | | | | | |
| L21M | N76D | S103A | V104I | M222S | K237R | Y263F | | | | | | |
| N76D | S103A | V104I | Q109R | M222S | | | | | | | | |
| N76D | S103A | V104I | Q109R | M222S | E271D | | | | | | | |
| G61R | N76D | S103A | V104I | M222S | | | | | | | | |
| N76D | S103A | V104I | Q137R | M222S | | | | | | | | |
| N76D | S103A | V104I | Q109R | M222S | N248S | | | | | | | |
| N76D | S103A | V104I | M222S | H249R | | | | | | | | |
| V68A | N76D | S103A | V104I | G159D | Q236H | Q245R | N261D | | | | | |
| V68A | N76D | S103A | V104I | S141N | G159D | Q236H | Q245R | T255S | | | | |
| V68A | N76D | S103A | V104I | G159D | Q236H | Q245R | R247H | | | | | |
| V68A | N76D | S103A | V104I | G159D | A174V | N204D | Q236H | Q245R | | | | |
| V68A | N76D | S103A | V104I | G159D | N204D | Q236H | Q245R | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V68A | N76D | S103A | V104I | A133V | G159D | N218D | Q236H | Q245R | | | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | | |
| V68A | N76D | S103A | V104I | G159D | A194I | V203A | Q236H | Q245R | | | | | |
| Q12R | N76D | S103A | V104I | M222S | Q245R | | | | | | | | |
| N76D | S103A | V104I | A232V | Q245R | | | | | | | | | |
| S24T | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N252K | | | | | | |
| V68A | N76D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | | | | |
| Q12R | N76D | S103A | I104T | M222S | V244I | Q245R | | | | | | | |
| Q12R | N76D | S103A | M222S | P210T | Q245R | | | | | | | | |
| Q12R | N76D | S103A | I104T | S130T | M222S | Q245R | | | | | | | |
| T22K | V68A | N76D | S103A | V104I | | | | | | | | | |
| V68A | N76D | S103A | V104I | N184D | | | | | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | | | |
| V68A | S103A | V104I | N140D | G159D | A232V | Q236H | Q245R | N252K | | | | | |
| N43S | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N252K | | | | | |
| N43K | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | | |
| N43D | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N252K | | | | | |
| V68A | S87G | S103A | V104I | G159D | A232V | Q236H | Q245R | N252K | R275S | | | | |
| Q12R | N76D | S103A | I104T | S130T | M222S | Q245R | N248S | L262M | | | | | |
| Q12R | N76D | S103A | I104T | S130T | A215V | M222S | Q245R | | | | | | |
| Q12R | N76D | S103A | I104T | S130T | M222S | V227A | Q245R | L262S | | | | | |
| Q12R | N76D | S103A | I104T | S130T | A215T | M222S | Q245R | | | | | | |
| Q12R | N76D | S103A | I104T | S130T | M222S | Q245R | N261D | | | | | | |
| N76D | S103A | I104T | S130T | M222S | Q245R | | | | | | | | |
| Q12R | N76D | S103A | I104T | S130T | N218D | M222S | Q245R | L262S | N269D | | | | |
| Q12R | S57P | N76D | S103A | I104T | S130T | M222S | Q245R | K251Q | | | | | |
| Q12R | N76D | S103A | I104T | S130T | R170S | N185D | M222S | N243D | Q245R | | | | |
| Q12R | N76D | S103A | I104T | S130T | M222S | Q245R | V268A | | | | | | |
| Q12R | N76D | S103A | I104T | S130T | M222S | P210S | Q245R | | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | | | | | | |
| V68A | S103A | V104I | N116D | G159D | A232V | Q236H | Q245R | | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | | | | | | |
| R10C | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | | |
| V68A | S103A | V104I | G159D | V203E | A232V | Q236H | Q245R | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V68A | S103A | V104I | G159D | A232V | Q236H | K237E | Q245R | | | | | |
| V68A | N76D | I79N | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | |
| V68A | S103A | V104I | G159D | N183D | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | A174V | Q206L | A232V | Q236H | Q245R | | | | |
| V68A | S103A | V104I | G159D | S188C | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | A230T | A232V | Q236H | Q245R | | | | | |
| V68A | A98T | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | A215T | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248S | | | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | |
| V68A | N76D | S103A | V104I | G159D | P210R | A232V | Q236H | Q245R | | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | | | | |
| N76D | S103A | V104I | A232V | Q236H | Q245R | L257V | | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | R275H | | | | |
| N76D | S103A | V104I | L257V | R275H | | | | | | | | |
| V68A | S103A | V104I | G159D | T224A | A232V | Q236H | Q245R | L257V | | | | |
| N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | | | | | |
| V68A | N76D | S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | | | | |
| V68A | N76D | S103A | V104I | G159D | G211R | A232V | Q236H | Q245R | | | | |
| V68A | N76D | S103A | V104I | G159D | G211V | A232V | Q236H | Q245R | | | | |
| Q12R | V68A | N76D | S103A | V104I | G159D | Y214L | A232V | Q236H | Q245R | | | |
| V68A | N76D | S103A | V104I | G159D | A215R | A232V | Q236H | Q245R | | | | |
| Q12R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | |
| G20R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | S259G | | | |
| V68A | S87R | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | T260V | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | N261G | | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | N261W | | | | |
| N76D | S103A | V104I | A232V | Q236H | S242P | Q245R | | | | | | |
| V68A | N76D | S103A | V104I | G159D | P210L | A232V | Q236H | Q245R | | | | |
| Q12R | A48V | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | |
| N76D | S103A | V104I | A232V | Q236H | Q245R | | | | | | | |
| N76D | S103A | V104I | G159D | V192F | A232V | Q236H | Q245R | | | | | |
| N76D | S103A | V104I | V147I | G159D | A232V | Q236H | Q245R | N248S | K251R | | | |
| Q12R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | A272S | | | |
| V68A | N76D | S103A | V104I | G159D | N183K | Q206L | A232V | Q236H | Q245R | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | S256R | | | | |

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| V68A | N76D | S103A | V104I | G159D | Q206R | A232V | Q236H | Q245R | | | | | |
| K27R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | |
| V68A | N76D | S103A | V104I | N116T | G159D | R170S | N185S | A232V | Q236H | Q245R | | | |
| G61E | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| N43D | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | S212P | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | S99N | G159D | N184D | A232V | Q236H | Q245R | N248D | N252K | | | |
| S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | | |
| V68A | S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | Q109R | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| Q20R | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | Y209F | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | N261D | | | | |
| V68A | S103A | V104I | G159D | N185D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | P210R | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | P210T | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | P210S | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | N185D | P210L | A232V | Q236H | Q245R | N248D | N252K | | | |
| V68A | S103A | V104I | G159D | P210L | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | S212A | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | S212E | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | T213E | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | T213S | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| V68A | A103V | V104I | G159D | T213E | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | T213G | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | A215V | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | A215R | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | S216T | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | S216V | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | S216C | A232V | Q236H | Q245R | N248D | N252K | | | | |
| G20A | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | N173D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | K251V | N252K | | | | |
| V68A | S103A | V104I | G159D | Q206R | A232V | Q236H | Q245R | N248D | N252K | | | | |

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| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252F | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252L | | | | |
| P55S | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252F | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | T255V | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | S256N | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | S256E | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | S256R | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | T260R | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | L257R | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | G258D | | | |
| I8V | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | N269D | | |
| V68A | S103A | V104I | N116S | G159D | A232V | Q236H | Q245R | N248D | N252K | T260F | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | N261R | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | N261D | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| V68A | S103A | V104I | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S103A | V104I | G159D | A232S | Q236H | Q245R | N248D | N252K | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236R | Q245R | N248D | N252K | | | | |
| N18S | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245V | N248D | N252K | | | | |
| V68A | N76D | S101T | S103A | V104I | G159D | T213R | N218S | A232V | Q236H | Q245R | T260A | |
| V68A | S103A | V104I | G159D | A228V | A232V | Q236H | Q245R | N248D | N252K | | | |
| T33S | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | |
| V68A | N76D | E89D | S103A | V104I | G159D | P210L | T213R | A232V | Q236H | Q245R | T260A | |
| Q61E | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | |
| S103A | V104I | G159D | V205I | P210I | A232V | Q236H | Q245R | | | | | |
| G61E | V68A | S103A | V104I | S130A | G159D | A232V | Q236H | Q245R | N248D | N252K | | |
| G61E | V68A | S103A | V104I | A133S | Q137R | G159D | A232V | Q236H | Q245R | N248D | N252K | |
| G61E | S103A | V104I | A133V | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248G | N252K | | | | |
| V68A | S103A | V104I | G159D | N218S | A232V | Q236H | Q245R | N248D | N252K | | | |
| G61E | V68A | S103A | V104I | G159D | S160V | A232V | Q236H | Q245R | N248D | N252K | | |
| S3L | G61E | V68A | N76D | S103A | V104I | A232V | Q236H | Q245R | N248D | N252K | | |
| G61E | V68A | S103A | V104I | G159D | S167F | A232V | Q236H | Q245R | N248D | N252K | | |
| G97E | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| A98D | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |

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| S99E | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S101E | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S101G | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| G102A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S103A | V104I | S106E | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S103A | V104I | Q109E | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | N261R | | | | | |
| S103A | V104I | Q109R | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| N62D | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S103A | V104I | G159D | N184D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S103A | V104I | G159D | S166D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S103A | V104I | G159D | L217E | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| G20R | N62D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | | |
| N62D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | | | |
| S103A | V104I | G159D | Q206R | L217E | A232V | Q236H | Q245R | N248D | N252K | | | | |
| N62D | S103A | V104I | G159D | Q206R | A232V | Q236H | Q245R | N248D | N252K | | | | |
| S103A | V104I | S130G | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S103A | V104I | P131V | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| K27N | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| T38G | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| T38A | N76D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | | | | |
| V68A | N76D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | E271G | | | |
| V68A | N76D | S103A | V104I | G159D | Y209W | T213R | A232V | Q236H | Q245R | T260A | | | |
| V68A | N76D | S103A | V104I | G159D | P210I | T213R | A232V | Q236H | Q245R | T260A | | | |
| V68A | N76D | S103A | V104I | G159D | V205I | T213R | A232V | Q236H | Q245R | T260A | | | |
| V68A | N76D | S103A | V104I | G159D | P210I | A232V | Q236H | Q245R | T260A | | | | |
| V68A | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | | | | | |
| N76D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | | | | | |
| V68A | S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | | | | | | |
| V68A | S103A | V104I | G159D | P210I | A232V | Q236H | Q245R | | | | | | |
| V68A | S103A | V104I | G159D | A230V | A232V | Q236H | Q245R | | | | | | |
| V68A | S103A | V104I | G159D | L126F | A232V | Q236H | Q245R | | | | | | |
| V68A | S103A | V104I | G159D | V205I | A232V | Q236H | Q245R | | | | | | |
| V68A | S103A | V104I | G159D | P210L | A232V | Q236H | Q245R | | | | | | |
| S103A | V104I | G159D | A230V | Q236H | Q245R | | | | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | T260A | | | | | | |

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| S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | | | |
| V68A | S103A | V104I | G159D | A174V | A232V | Q236H | Q245R | L257V | | | | |
| V68A | S103A | V104I | G159D | A194S | A232V | Q236H | Q245R | L257V | | | | |
| V68A | S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | L257V | | | | |
| S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | | | | | | |
| V68A | N76D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | N261W | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | N261W | | | | |
| S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | | | | | |
| S103A | V104I | G159D | P210I | A232V | Q236H | Q245R | N248D | N252K | | | | |
| S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | L257V | | | | | |
| V68A | N76D | S103A | V104I | G159D | P210L | T213R | A232V | Q236H | Q245R | T260A | | |
| Q12R | S103A | V104I | G159D | Y209W | T213R | A232V | Q236H | Q245R | T260A | | | |
| S103A | V104I | Y209W | A232V | Q236H | Q245R | L257V | | | | | | |
| S103A | V104I | G159D | V205I | P210I | T213R | A232V | Q236H | Q245R | T260A | | | |
| S103A | V104I | G159D | V205I | Y209W | A232V | Q236H | Q245R | T260A | | | | |
| V68A | S103A | V104I | G159D | V205I | Y209W | P210I | A232V | Q236H | Q245R | | | |
| S103A | V104I | G159D | V205I | Y209W | P210I | A232V | Q236H | Q245R | L257V | | | |
| S103A | V104I | G159D | V205I | Y209W | A232V | Q236H | Q245R | L257V | | | | |
| V68A | S103A | V104I | G159D | V205I | Y209W | P210I | A232V | Q236H | Q245R | T260A | | |
| S103A | V104I | G159D | V205I | Y209W | P210I | A232V | Q236H | Q245R | | | | |
| S103A | V104I | G159D | Y209W | P210I | A232V | Q236H | Q245R | | | | | |
| S103A | V104I | G159D | V205I | P210I | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | S128L | G159D | A232V | Q236H | Q245R | | | | | |
| A48V | S103A | V104I | G159D | A230V | Q236H | Q245R | | | | | | |
| A48V | V68A | S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | | | | |
| A48V | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| A48V | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | N261W | | | |
| G102A | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | | |
| Q12R | G102A | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | |
| S101G | G102A | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | |
| A98L | G102A | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | |
| G102A | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | | |
| S103A | V104I | P131V | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| S103A | V104I | G159D | N184S | A232V | Q236H | Q245R | N248D | N252K | | | | |
| S103A | V104I | G159D | N184G | A232V | Q236H | Q245R | N248D | N252K | | | | |
| S103A | V104I | G159D | A232V | Q236H | V244T | Q245R | N248D | N252K | | | | |

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| S103A | V104I | G159D | A232V | Q236H | V244A | Q245R | N248D | N252K | | | | |
| N62D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | S256R | | |
| Q12R | N62D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| S101G | S103A | V104I | G159D | N185D | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | G159D | Q206E | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | G159D | T213Q | A232V | Q236H | Q245R | N248D | N252K | | | |
| A98L | G102A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | G102A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| A98L | G102A | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | |
| A98L | G102A | S103A | V104I | G159D | S212G | A232V | Q236H | N248D | N252K | | | |
| N62D | S103A | V104I | Q109R | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| N62D | S103A | V104I | G159D | S212G | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| N62D | S101G | S103A | V104I | G159D | S212G | T213R | A232V | Q236H | Q245R | N248D | N252K | |
| S103A | V104I | G159D | A232V | Q245R | N248D | N252K | | | | | | |
| S103A | V104I | G159D | A230V | Q245R | | | | | | | | |
| N62D | S103A | V104I | S130G | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| S101G | S103A | V104I | S130G | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | S128G | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | S128L | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| N62D | S101G | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| N62D | S103A | V104I | S128G | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| N62D | S103A | V104I | S128L | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| S101G | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | T260A | | | |
| S101G | S103A | V104I | P131V | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| A98V | S101G | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| S99G | S101G | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | | |
| | | | | | | | | | | | | |
| S101G | S103A | V104I | G159D | V209W | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | G159D | P210I | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | G159D | V205I | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | G159D | A230V | Q236H | Q245R | | | | | | |
| S101G | S103A | V104I | G159D | A194P | A232V | Q236H | Q245R | N248D | N252K | | | |
| N76D | S101G | S103A | V104I | G159D | A194P | A232V | Q236H | Q245R | N248D | N252K | | |
| S101G | S103A | V104I | G159D | A230V | A232V | Q236H | Q245R | N248D | N252K | | | |
| N62D | S103A | V104I | G159D | N185D | Q206E | T213R | A232V | Q236H | Q245R | N248D | N252K | E271Q |

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table I except for the following substitution sets of Table III:

Table III

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 76 | 103 | 104 | 259 | | | | | | | |
| 76 | 86 | 103 | 104 | | | | | | | |
| 76 | 103 | 104 | 130 | | | | | | | |
| 76 | 99 | 103 | 104 | 204 | | | | | | |
| 76 | 103 | 104 | 242 | | | | | | | |
| 76 | 103 | 104 | 104 | 182 | 198 | | | | | |
| 21 | 76 | 103 | 104 | 182 | | | | | | |
| 76 | 103 | 104 | 119 | 137 | | | | | | |
| 76 | 103 | 104 | 173 | 222 | | | | | | |
| 61 | 76 | 103 | 104 | 222 | | | | | | |
| 68 | 76 | 103 | 104 | 116 | 159 | 170 | 185 | 232 | 236 | 245 |

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table IV:

Table IV

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 76 | 103 | 104 | 222 | 245 | | | | | | | |
| 76 | 103 | 104 | 222 | 249 | | | | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 252 | | | | |
| 68 | 76 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 260 | | |
| 22 | 68 | 76 | 103 | 104 | | | | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 140 | 159 | 232 | 236 | 245 | 252 | | | |
| 43 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 252 | | | |
| 43 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | | | | |
| 12 | 76 | 103 | 104 | 130 | 222 | 245 | 261 | | | | |
| 76 | 103 | 104 | 130 | 222 | 245 | | | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 257 | | | | |
| 68 | 76 | 103 | 104 | 159 | 210 | 232 | 236 | 245 | | | |
| 68 | 103 | 104 | 159 | 224 | 232 | 236 | 245 | 257 | | | |
| 76 | 103 | 104 | 159 | 232 | 236 | 245 | 257 | | | | |
| 68 | 76 | 103 | 104 | 159 | 211 | 232 | 236 | 245 | | | |
| 12 | 68 | 76 | 103 | 104 | 159 | 214 | 232 | 236 | 245 | | |
| 68 | 76 | 103 | 104 | 159 | 215 | 232 | 236 | 245 | | | |
| 12 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | |
| 20 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 259 | | |
| 68 | 76 | 87 | 103 | 104 | 159 | 232 | 236 | 245 | 260 | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 261 | | | |
| 12 | 48 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | |
| 76 | 103 | 104 | 159 | 192 | 232 | 236 | 245 | | | | |
| 76 | 103 | 104 | 147 | 159 | 232 | 236 | 245 | 248 | 251 | | |
| 12 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 272 | | |
| 68 | 76 | 103 | 104 | 159 | 183 | 206 | 232 | 236 | 245 | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 256 | | | |
| 68 | 76 | 103 | 104 | 159 | 206 | 232 | 236 | 245 | | | |
| 27 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | |
| 68 | 103 | 104 | 159 | 212 | 232 | 236 | 245 | 248 | 252 | | |
| 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 68 | 103 | 104 | 159 | 209 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 109 | 159 | 232 | 236 | 245 | 248 | 252 | | |
| 20 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 159 | 209 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 159 | 210 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 159 | 212 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 213 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 215 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 159 | 216 | 232 | 236 | 245 | 248 | 252 | | |
| 20 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 255 | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 256 | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 260 | | |
| 68 | 103 | 104 | 159 | 228 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 76 | 89 | 103 | 104 | 159 | 210 | 213 | 232 | 236 | 245 | 260 |
| 68 | 103 | 104 | 159 | 218 | 232 | 236 | 245 | 248 | 252 | | |

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table V:

Table V

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| V68A | S103A | V104I | G159D | A228V | A232V | Q236H | Q245R | N248D | N252K | |
| V68A | S103A | V104I | G159D | N218S | A232V | Q236H | Q245R | N248D | N252K | |
| G20R | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | |
| V68A | N76D | E89D | S103A | V104I | G159D | P210L | T213R | A232V | Q236H | Q245R | T260A |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | S256R | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | T260R | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | T255V | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | S256N | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252L | | |
| V68A | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | |
| V68A | S103A | V104I | G159D | A215V | A232V | Q236H | Q245R | N248D | N252K | |
| V68A | S103A | V104I | G159D | A215R | A232V | Q236H | Q245R | N248D | N252K | |
| V68A | S103A | V104I | G159D | S216T | A232V | Q236H | Q245R | N248D | N252K | |
| V68A | S103A | V104I | G159D | S216V | A232V | Q236H | Q245R | N248D | N252K | |
| V68A | S103A | V104I | T213S | A232V | Q236H | Q245R | N248D | N252K | | |
| V68A | S103A | V104I | G159D | P210L | A232V | Q236H | Q245R | N248D | N252K | |
| V68A | S103A | V104I | G159D | S212C | A232V | Q236H | Q245R | N248D | N252K | |
| V68A | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | |
| S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| V68A | S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | N248D | N252K | |
| V68A | S103A | V104I | Q109R | G159D | A232V | Q236H | Q245R | N248D | N252K | |
| G20R | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | |
| V68A | S103A | V104I | G159D | Y209F | A232V | Q236H | Q245R | N248D | N252K | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Q12R | N76D | S103A | I104T | S130T | M222S | Q245R | N261D | | | | | |
| N76D | S103A | I104T | S130T | M222S | Q245R | | | | | | | |
| N76D | S103A | V104I | M222S | H249R | | | | | | | | |
| N76D | S103A | V104I | M222S | Q245R | | | | | | | | |
| N76D | S103A | V104I | G159D | Y192F | A232V | Q236H | Q245R | | | | | |
| N76D | S103A | V104I | V147I | G159D | A232V | Q236H | Q245R | N248S | K251R | | | |
| Q12R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | A272S | | | |
| V68A | N76D | S103A | V104I | G159D | N183K | Q206L | A232V | Q236H | Q245R | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | S256R | | | | |
| V68A | N76D | S103A | V104I | G159D | Q206R | A232V | Q236H | Q245R | | | | |
| K27R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | |
| Q12R | A48V | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | N261W | | | | |
| V68A | N76D | S103A | V104I | G159D | G211R | A232V | Q236H | Q245R | | | | |
| V68A | N76D | S103A | V104I | G159D | G211V | A232V | Q236H | Q245R | | | | |
| Q12R | V68A | N76D | S103A | V104I | G159D | Y214L | A232V | Q236H | Q245R | | | |
| V68A | N76D | S103A | V104I | G159D | A215R | A232V | Q236H | Q245R | | | | |
| Q12R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | |
| G20R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | S259G | | | |
| V68A | N76D | S87R | S103A | V104I | G159D | A232V | Q236H | Q245R | T260V | | | |
| N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | | | | | |
| V68A | N76D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | | | |
| T22K | V68A | N76D | S103A | V104I | | | | | | | | |
| V68A | N76D | S103A | V104I | G159D | P210R | A232V | Q236H | Q245R | | | | |
| V68A | S103A | V104I | G159D | S212F | A232V | Q236H | Q245R | N248D | N252K | | | |
| V68A | S103A | V104I | G159D | T224A | A232V | Q236H | Q245R | L257V | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N252S | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N252K | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | | |
| V68A | S103A | V104I | N140D | G159D | A232V | Q236H | Q245R | N252K | | | | |
| N43S | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N252K | | | | |
| N43K | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | |
| N43D | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N252K | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | | | | | |

A highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

12/102/103/104/159/212/232/236/245/248/252;
12/76/103/104/130/222/245/261;
12/76/103/104/222/245;
61/68/103/104/159/232/236/245/248/252;
62/103/104/109/159/213/232/236/245/248/252;
62/101/103/104/159/212/213/232/236/245/248/252;
62/103/104/130/159/213/232/236/245/248/252;
68/103/104/159/185/232/236/245/248/252;
68/103/104/159/185/210/232/236/245/248/252;
68/103/104/159/230/232/236/245;
68/103/104/232/236/245/248/257/275;
68/103/104/159/232/236/245/248/252;
68/76/103/104/159/236;
68/76/103/104/159/232/236/245;
68/103/104/159/232/236/245;
68/76/103/104/159/211/232/236/245;
68/103/104/159/210/232/236/245;
68/76/103/104/159/213/232/236/245/260;
68/76/103/104/159/210/232/236/245/260;
68/103/104/159/183/232/236/245/248/252;
68/103/104/232/236/245/257/275;
76/103/222/245;
76/103/104/159/232/236/245;
76/103/104/159/213/232/236/245/260;
76/103/104/131/159/232/236/245/248/252;
98/102/103/104/159/212/232/236/245/248/252;
101/103/104/159/232/236/245/248/252;
103/104/159/232/236/245;
103/104/159/205/209/232/236/245/257
103/104/159/205/209/210/232/236/245/257;
103/104/159/217/232/236/245/248/252;
103/104/159/230/236/245;
103/104/159/248/252/270;
103/104/159/205/209/232/236/245; and
12/76/103/104/130/170/185/222/243/245;
12/76/103/104/130/222/245;
62/103/104/159/213/232/236/245/248/252;
62/103/104/159/232/236/245/248/252;
68/103/104/159/232/236/245/248/252/270;
68/103/104/159/210/232/236/245/248/252;
68/103/104/159/213/232/236/245/248/252;
68/76/103/104/159/209/232/236/245;
68/103/104/213/232/236/245/248/252;
68/103/104/159/209/232/236/245;
68/76/103/104/159/236/245;
68/103/104/159/232/236/245/252;
68/103/104/159/232/236/245/257;
68/76/103/104/159/215/232/236/245;
68/103/104/159/213/232/236/245/260;
68/103/104/159/236;
68/103/104/159/236/245;
68/76/103/104/159/236/245;
68/103/104/159/213/232/236/245;
76/103/104/222/245;
76/103/104/159;
97/103/104/159/232/236/245/248/252;
98/103/104/159/232/236/245/248/252;
102/103/104/159/232/236/245/248/252;
103/104/159/232/236/245/248/252;
103/104/159/232/245/248/252;
103/104/159/213/232/236/245/248/252;
103/104/130/159/232/236/245/248/252;
103/104/159/236/245;
103/104/131/159/232/236/245/248/252;
103/104/159/232/236/245/257.

A more highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

12R/76D/103A/104T/130T/222S/245R;
12R/76D/103A/104I/222S/245R;
12R/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K;
12R/76D/103A/104T/130G/222S/245R/261D;
12R/76D/103A/104T/130G/170S/185D/222S/243D/245R;
61E/68A/103A/104I/159D/232V/236H/245R/248D/252K;
62D/103A/104I/109R/159D/213R/232V/236H/245R/248D/252K;
62D/103A/104I/159D/213R/232V/236H/245R/248D/252K;
62D/103A/104I/159D/232V/236H/245R/248D/252K;
62D/103A/104I/130G/159D/213R/232V/236H/245R/248D/252K;
62D/101G/103A/104I/159D/212G/213R/232V/236H/245R/248D/252K;
68A/103A/104I/159D/232V/236H/245R/248D/252K/270A;
68A/76D/103A/104I/159D/213R/232V/236H/245R/260A;
68A/103A/104I/159D/236H;
68A/103A/104I/159D/236H/245R;
68A/76D/103A/104I/159D/210I/232V/236H/245R/260A;
68A/103A/104I/159D/183D/232V/236H/245R/248D/252K;
68A/103A/104I/159D/209W/232V/236H/245R;
68A/76D/103A/104I/159D/211R/232V/236H/245R;
68A/76D/103A/104I/159D/215R/232V/236H/245R;
68A/103A/104I/159D/213R/232V/236H/245R/260A;
68A/76D/103A/104I/159D/236H;
68A/76D/103A/104I/159D/236H/245R;
68A/76D/103A/104I/159D/232V/236H/245R;
68A/103A/104I/159D/232V/236H/245R/252K;
68A/103A/104I/159D/232V/236H/245R;
68A/103A/104I/159D/232V/236H/245R/257V;
68A/103A/104I/159D/185D/232V/236H/245R/248D/252K;
68A/103A/104I/159D/210L/232V/236H/245R/248D/252K;
68A/103A/104I/159D/185D/210L/232V/236H/245R/248D/252K;
68A/103A/104I/159D/213E/232V/236H/245R/248D/252K;
68A/103A/104I/159D/230V/232V/236H/245R;

68A/76D/103A/104I/159D/209W/232V/236H/245R;
68A/103A/104I/232V/236H/245R/248D/257V/275H;
68A/103A/104I/232V/236H/245R/257V/275H;
68A/103A/104I/213E/232V/236H/245R/248D/252K;
68A/103A/104I/159D/232V/236H/245R/248D/252K;
68A/103A/104I/159D/210I/232V/236H/245R;
68A/103A/104I/159D/210L/232V/236H/245R;
68A/103A/104I/159D/213G/232V/236H/245R;
76D/103A/222S/245R;
76D/103A/104I/222S/245R;
76D/103A/104I/159D/232V/236H/245R;
76D/103A/104I/159D;
76D/103A/104I/131V/159D/232V/236H/245R/248D/252K;
76D/103A/104I/159D/213R/232V/236H/245R/260A;
97E/103A/104I/159D/232V/236H/245R/248D/252K;
98L/103A/104I/159D/232V/236H/245R/248D/252K;
98L/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K;
101G/103A/104I/159D/232V/236H/245R/248D/252K;
102A/103A/104I/159D/232V/236H/245R/248D/252K;
103A/104I/159D/232V/236H/245R/248D/252K;
103A/104I/159D/213R/232V/236H/245R/248D/252K;
103A/104I/130G/159D/232V/236H/245R/248D/252K;
103A/104I/159D/230V/236H/245R;
103A/104I/159D/217E/232V/236H/245R/248D/252K;
103A/104I/159D/236H/245R;
103A/104I/159D/248D/252K/270V;
103A/104I/159D/232V/236H/245R;
103A/104I/159D/205I/209W/232V/236H/245R;
103A/104I/159D/232V/236H/245R/257V;
103A/104I/159D/205I/209W/232V/236H/245R/257V;
103A/104I/131V/159D/232V/236H/245R/248D/252K;
103A/104I/159D/205I/209W/210I/232V/236H/245R/257V; and
103A/104I/159D/232V/245R/248D/252K.

An even more highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

12/76/103/104/130/222/245/261;
62/103/104/159/232/236/245/248/252;
62/103/104/159/213/232/236/245/248/252;
62/101/103/104/159/212/213/232/236/245/248/252;
68/103/104/159/232/236/245;
68/103/104/159/230/232/236/245;
68/103/104/159/209/232/236/245;
68/103/104/159/232/236/245/257;
68/76/103/104/159/213/232/236/245/260;
68/103/104/159/213/232/236/245/248/252;
68/103/104/159/183/232/236/245/248/252;
68/103/104/159/185/232/236/245/248/252;
68/103/104/159/185/210/232/236/245/248/252;
68/103/104/159/210/232/236/245/248/252;
68/103/104/159/213/232/236/245;
98/103/104/159/232/236/245/248/252;
98/102/103/104/159/212/232/236/245/248/252;
101/103/104/159/232/236/245/248/252;
102/103/104/159/232/236/245/248/252;
103/104/159/230/236/245;
103/104/159/232/236/245/248/252;
103/104/159/217/232/236/245/248/252;
103/104/130/159/232/236/245/248/252;
103/104/131/159/232/236/245/248/252;
103/104/159/213/232/236/245/248/252; and
103/104/159/232/236/245.

The most highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

12R/76D/103A/104T/130T/222S/245R/261D;
62D/103A/104I/159D/232V/236H/245R/248D/252K;
62D/103A/104I/159D/213R/232V/236H/245R/248D/252K;
68A/103A/104I/159D/209W/232V/236H/245R;
68A/76D/103A/104I/159D/213R/232V/236H/245R/260A;
68A/103A/104I/159D/213E/232V/236H/245R/248D/252K;
68A/103A/104I/159D/183D/232V/236H/245R/248D/252K;

68A/103A/104I/159D/232V/236H/245R;
68A/103A/104I/159D/230V/232V/236H/245R;
68A/103A/104I/159D/232V/236H/245R/257V;
68A/103A/104I/159D/213G/232V/236H/245R/248D/252K;
68A/103A/104I/159D/185D/232V/236H/245R/248D/252K;
68A/103A/104I/159D/185D/210L/232V/236H/245R/248D/252K;
68A/103A/104I/159D/210L/232V/236H/245R/248D/252K;
68A/103A/104I/159D/213G/232V/236H/245R;
98L/103A/104I/159D/232V/236H/245R/248D/252K;
98L/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K;
101G/103A/104I/159D/232V/236H/245R/248D/252K;
102A/103A/104I/159D/232V/236H/245R/248D/252K;
103A/104I/159D/230V/236H/245R;
103A/104I/159D/232V/236H/245R/248D/252K;
103A/104I/159D/217E/232V/236H/245R/248D/252K;
103A/104I/130G/159D/232V/236H/245R/248D/252K;
103A/104I/131V/159D/232V/236H/245R/248D/252K;
103A/104I/159D/213R/232V/236H/245R/248D/252K; and
103A/104I/159D/232V/236H/245R.

In another preferred embodiment, the protease variants which are the protease enzymes useful in the cleaning compositions of the present invention comprise protease variants including a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin.

While any combination of the above listed amino acid substitutions may be employed, the preferred protease variant enzymes useful for the present invention comprise the substitution, deletion or insertion of amino acid residues in the following combinations:

(1) a protease variant including substitutions of the amino acid residues at position 62 and at one or more of the following positions 103, 104, 109, 159, 213, 232, 236, 245, 248 and 252;

(2) a protease variant including substitutions of the amino acid residues at position 212 and at one or more of the following positions 12, 98, 102, 103, 104, 159, 232, 236, 245, 248 and 252;

(3) a protease variant including substitutions of the amino acid residues at position 230 and at one or more of the following positions 68, 103, 104, 159, 232, 236 and 245;

(4) a protease variant including substitutions of the amino acid residues at position 232 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;

(5) a protease variant including substitutions of the amino acid residues at position 232 and at one or more of the following positions 103, 104, 236 and 245;

(6) a protease variant including substitutions of the amino acid residues at position 232 and 103 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;

(7) a protease variant including substitutions of the amino acid residues at position 232 and 104 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;

(8) a protease variant including substitutions of the amino acid residues at position 232 and 236 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;

(9) a protease variant including substitutions of the amino acid residues at position 232 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;

(10) a protease variant including substitutions of the amino acid residues at position 232, 103, 104, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;

(11) a protease variant including substitutions of the amino acid residues at position 252 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;

(12) a protease variant including substitutions of the amino acid residues at position 252 and at one or more of the following positions 103, 104, 236 and 245;

(13) a protease variant including substitutions of the amino acid residues at positions 252 and 103 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;

(14) a protease variant including substitutions of the amino acid residues at positions 252 and 104 and at one or more of the following positions 12, 61, 62, 68, 97, 98,

101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;

(15) a protease variant including substitutions of the amino acid residues at positions 252 and 236 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;

(16) a protease variant including substitutions of the amino acid residues at positions 252 and 245 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;

(17) a protease variant including substitutions of the amino acid residues at positions 252, 103, 104, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270; and

(18) a protease variant including substitutions of the amino acid residues at position 257 and at one or more of the following positions 68, 103, 104, 205, 209, 210, 232, 236, 245 and 275.

A more preferred protease variant useful in the cleaning compositions of the present invention include a substitution set (one substitution set per row in the following Table VI) selected from the group consisting of:

Table VI

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 76 | 103 | 104 | 212 | 271 | | | | | | | | |
| 76 | 103 | 104 | 252 | 261 | | | | | | | | |
| 76 | 103 | 104 | 212 | 258 | | | | | | | | |
| 4 | 76 | 103 | 104 | 159 | 217 | 252 | | | | | | |
| 12 | 62 | 76 | 103 | 104 | 159 | | | | | | | |
| 76 | 103 | 104 | 212 | 268 | 271 | | | | | | | |
| 76 | 87 | 103 | 104 | 212 | 271 | | | | | | | |
| 76 | 103 | 104 | 212 | 245 | 271 | | | | | | | |
| 76 | 103 | 104 | 134 | 141 | 212 | 271 | | | | | | |
| 76 | 103 | 104 | 212 | 236 | 243 | 271 | | | | | | |
| 20 | 62 | 76 | 103 | 104 | | | | | | | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | | | |
| 76 | 103 | 104 | 232 | 245 | | | | | | | | |
| 24 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 252 | | | | | |
| 68 | 76 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 260 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | | | | | | |
| 68 | 103 | 104 | 140 | 159 | 232 | 236 | 245 | 252 | | | | |
| 43 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 252 | | | | |
| 43 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | | | | | |
| 43 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 252 | | | | |
| 68 | 87 | 103 | 104 | 159 | 232 | 236 | 245 | 252 | 275 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 257 | | | | | |
| 68 | 103 | 104 | 116 | 159 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | | | | | |
| 10 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 203 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 237 | 245 | | | | | |
| 68 | 76 | 79 | 103 | 104 | 159 | 232 | 236 | 245 | | | | |
| 68 | 103 | 104 | 159 | 183 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 174 | 206 | 232 | 236 | 245 | | | | |
| 68 | 103 | 104 | 159 | 188 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 230 | 232 | 236 | 245 | | | | | |
| 68 | 98 | 103 | 104 | 159 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 215 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | | | | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | | | |
| 68 | 76 | 103 | 104 | 159 | 210 | 232 | 236 | 245 | | | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 257 | | | | |
| 76 | 103 | 104 | 232 | 236 | 245 | 257 | | | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 257 | 275 | | | | |
| 76 | 103 | 104 | 257 | 275 | | | | | | | | |
| 68 | 103 | 104 | 159 | 224 | 232 | 236 | 245 | 257 | | | | |
| 76 | 103 | 104 | 159 | 232 | 236 | 245 | 257 | | | | | |
| 68 | 76 | 103 | 104 | 159 | 209 | 232 | 236 | 245 | | | | |
| 68 | 76 | 103 | 104 | 159 | 211 | 232 | 236 | 245 | | | | |
| 12 | 68 | 76 | 103 | 104 | 159 | 214 | 232 | 236 | 245 | | | |
| 68 | 76 | 103 | 104 | 159 | 215 | 232 | 236 | 245 | | | | |
| 12 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 26 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 259 | | | |
| 68 | 87 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 260 | | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 261 | | | | |
| 76 | 103 | 104 | 232 | 236 | 242 | 245 | | | | | | |
| 68 | 76 | 103 | 104 | 159 | 210 | 232 | 236 | 245 | | | | |
| 12 | 48 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | |
| 76 | 103 | 104 | 232 | 236 | 245 | | | | | | | |
| 76 | 103 | 104 | 159 | 192 | 232 | 236 | 245 | | | | | |
| 76 | 103 | 104 | 147 | 159 | 232 | 236 | 245 | 248 | 251 | | | |
| 12 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 272 | | | |
| 68 | 76 | 103 | 104 | 159 | 183 | 206 | 232 | 236 | 245 | | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 256 | | | | |
| 68 | 76 | 103 | 104 | 159 | 206 | 232 | 236 | 245 | | | | |
| 27 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | | |
| 68 | 76 | 103 | 104 | 116 | 159 | 170 | 185 | 232 | 236 | 245 | | |
| 61 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 43 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 212 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 99 | 159 | 184 | 232 | 236 | 245 | 248 | 252 | | |
| 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | | |
| 68 | 103 | 104 | 159 | 209 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 109 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 20 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 209 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 261 | | | |
| 68 | 103 | 104 | 159 | 185 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 210 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 185 | 210 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 159 | 212 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 213 | 232 | 236 | 245 | 248 | 252 | | | | |
| 68 | 103 | 104 | 159 | 215 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 216 | 232 | 236 | 245 | 248 | 252 | | | |
| 20 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 173 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 251 | 252 | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 68 | 103 | 104 | 159 | 206 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 55 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 255 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 256 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 260 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 257 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 258 | | | |
| 8 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 269 | | |
| 68 | 103 | 104 | 116 | 159 | 232 | 236 | 245 | 248 | 252 | 260 | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 261 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 261 | | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 232 | 236 | 245 | 248 | 252 | | | | | |
| 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 18 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 68 | 76 | 101 | 103 | 104 | 159 | 213 | 218 | 232 | 236 | 245 | 260 | |
| 68 | 103 | 104 | 159 | 228 | 232 | 236 | 245 | 248 | 252 | | | |
| 33 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 76 | 89 | 103 | 104 | 159 | 210 | 213 | 232 | 236 | 245 | 260 | |
| 61 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | |
| 103 | 104 | 159 | 205 | 210 | 232 | 236 | 245 | | | | | |
| 61 | 68 | 103 | 104 | 130 | 159 | 232 | 236 | 245 | 248 | 252 | | |
| 61 | 68 | 103 | 104 | 133 | 137 | 159 | 232 | 236 | 245 | 248 | 252 | |
| 61 | 103 | 104 | 133 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 68 | 103 | 104 | 159 | 218 | 232 | 236 | 245 | 248 | 252 | | | |
| 61 | 68 | 103 | 104 | 159 | 160 | 232 | 236 | 245 | 248 | 252 | | |
| 3 | 61 | 68 | 76 | 103 | 104 | 232 | 236 | 245 | 248 | 252 | | |
| 61 | 68 | 103 | 104 | 159 | 167 | 232 | 236 | 245 | 248 | 252 | | |
| 97 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 98 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 99 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 101 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 102 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 103 | 104 | 106 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 103 | 104 | 109 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 261 | | | | |
| 62 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 103 | 104 | 159 | 184 | 232 | 236 | 245 | 248 | 252 | | | | |
| 103 | 104 | 159 | 166 | 232 | 236 | 245 | 248 | 252 | | | | |
| 103 | 104 | 159 | 217 | 232 | 236 | 245 | 248 | 252 | | | | |
| 20 | 62 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 62 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | | |
| 103 | 104 | 159 | 206 | 217 | 232 | 236 | 245 | 248 | 252 | | | |
| 62 | 103 | 104 | 159 | 206 | 232 | 236 | 245 | 248 | 252 | | | |
| 103 | 104 | 130 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 103 | 104 | 131 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 27 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 38 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 38 | 76 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 260 | | | |
| 68 | 76 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 260 | 271 | | |
| 68 | 76 | 103 | 104 | 159 | 209 | 213 | 232 | 236 | 245 | 260 | | |
| 68 | 76 | 103 | 104 | 159 | 210 | 213 | 232 | 236 | 245 | 260 | | |
| 68 | 76 | 103 | 104 | 159 | 205 | 213 | 232 | 236 | 245 | 260 | | |
| 68 | 76 | 103 | 104 | 159 | 210 | 232 | 236 | 245 | 260 | | | |
| 68 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 260 | | | | |
| 76 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 260 | | | | |
| 68 | 103 | 104 | 159 | 209 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 210 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 230 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 126 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 205 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 159 | 210 | 232 | 236 | 245 | | | | | |
| 103 | 104 | 159 | 230 | 236 | 245 | | | | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 260 | | | | | |
| 103 | 104 | 159 | 232 | 236 | 245 | | | | | | | |
| 68 | 103 | 104 | 159 | 174 | 232 | 236 | 245 | 257 | | | | |
| 68 | 103 | 104 | 159 | 194 | 232 | 236 | 245 | 257 | | | | |
| 68 | 103 | 104 | 159 | 209 | 232 | 236 | 245 | 257 | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 103 | 104 | 159 | 232 | 236 | 245 | 257 | | | | | | |
| 68 | 76 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 260 | 261 | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 257 | 261 | | | | |
| 103 | 104 | 159 | 213 | 232 | 236 | 245 | 260 | | | | | |
| 103 | 104 | 159 | 210 | 232 | 236 | 245 | 248 | 252 | | | | |
| 103 | 104 | 159 | 209 | 232 | 236 | 245 | 257 | | | | | |
| 68 | 76 | 103 | 104 | 159 | 210 | 213 | 232 | 236 | 245 | 260 | | |
| 12 | 103 | 104 | 159 | 209 | 213 | 232 | 236 | 245 | 260 | | | |
| 103 | 104 | 209 | 232 | 236 | 245 | 257 | | | | | | |
| 103 | 104 | 159 | 205 | 210 | 213 | 232 | 236 | 245 | 260 | | | |
| 103 | 104 | 159 | 205 | 209 | 232 | 236 | 245 | 260 | | | | |
| 68 | 103 | 104 | 159 | 205 | 209 | 210 | 232 | 236 | 245 | | | |
| 103 | 104 | 159 | 205 | 209 | 210 | 232 | 236 | 245 | 257 | | | |
| 103 | 104 | 159 | 205 | 209 | 232 | 236 | 245 | 257 | | | | |
| 68 | 103 | 104 | 159 | 205 | 209 | 210 | 232 | 236 | 245 | 260 | | |
| 103 | 104 | 159 | 205 | 209 | 210 | 232 | 236 | 245 | | | | |
| 103 | 104 | 159 | 209 | 210 | 232 | 236 | 245 | | | | | |
| 103 | 104 | 159 | 205 | 210 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 128 | 159 | 232 | 236 | 245 | | | | | |
| 48 | 103 | 104 | 159 | 230 | 236 | 245 | | | | | | |
| 48 | 68 | 103 | 104 | 159 | 209 | 232 | 236 | 245 | | | | |
| 48 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 48 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 257 | 261 | | | |
| 102 | 103 | 104 | 159 | 212 | 232 | 236 | 245 | 248 | 252 | | | |
| 12 | 102 | 103 | 104 | 159 | 212 | 232 | 236 | 245 | 248 | 252 | | |
| 101 | 102 | 103 | 104 | 159 | 212 | 232 | 236 | 245 | 248 | 252 | | |
| 98 | 102 | 103 | 104 | 159 | 212 | 232 | 236 | 245 | 248 | 252 | | |
| 102 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | | |
| 103 | 104 | 131 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 103 | 104 | 159 | 184 | 232 | 236 | 245 | 248 | 252 | | | | |
| 103 | 104 | 159 | 232 | 236 | 244 | 245 | 248 | 252 | | | | |
| 62 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | 256 | | |
| 12 | 62 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 101 | 103 | 104 | 159 | 185 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 103 | 104 | 159 | 206 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 98 | 102 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 102 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 98 | 102 | 103 | 104 | 159 | 212 | 232 | 236 | 245 | 248 | 252 | | |
| 98 | 102 | 103 | 104 | 159 | 212 | 232 | 236 | 248 | 252 | | | |
| 62 | 103 | 104 | 109 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 62 | 103 | 104 | 159 | 212 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 62 | 101 | 103 | 104 | 159 | 212 | 213 | 232 | 236 | 245 | 248 | 252 | |
| 103 | 104 | 159 | 232 | 245 | 248 | 252 | | | | | | |
| 103 | 104 | 159 | 230 | 245 | | | | | | | | |
| 62 | 103 | 104 | 130 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 101 | 103 | 104 | 130 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 103 | 104 | 128 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 62 | 101 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 62 | 103 | 104 | 128 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 62 | 103 | 104 | 128 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 101 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 260 | | | |
| 101 | 103 | 104 | 131 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 98 | 101 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 99 | 101 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 103 | 104 | 159 | 212 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 103 | 104 | 159 | 209 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 103 | 104 | 159 | 210 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 103 | 104 | 159 | 205 | 232 | 236 | 245 | 248 | 252 | | | |
| 101 | 103 | 104 | 159 | 230 | 236 | 245 | | | | | | |
| 101 | 103 | 104 | 159 | 194 | 232 | 236 | 245 | 248 | 252 | | | |
| 76 | 101 | 103 | 104 | 159 | 194 | 232 | 236 | 245 | 248 | 252 | | |
| 101 | 103 | 104 | 159 | 230 | 232 | 236 | 245 | 248 | 252 | | | |
| 62 | 103 | 104 | 159 | 185 | 206 | 213 | 232 | 236 | 245 | 248 | 252 | 271 |

An even more preferred protease variant useful in the cleaning compositions of the present invention include a substitution set (one substitution set per row in the following Table VII) selected from the group consisting of:

Table VII

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N76D | S103A | V104I | S212P | E271V | | | | | | | |
| N76D | S103A | V104I | N252K | N261Y | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N76D | S103A | V104I | S212P | G258R | | | | | | | | |
| V4E | N76D | S103A | V104I | G159D | L217E | N252D | | | | | | |
| Q12H | N62H | N76D | S103A | V104I | G159D | | | | | | | |
| N76D | S103A | V104I | S212P | V268F | E271V | | | | | | | |
| N76D | S87R | S103A | V104I | S212P | E271V | | | | | | | |
| N76D | S103A | V104I | S212P | Q245L | E271V | | | | | | | |
| N76D | S103A | V104I | T134S | S141N | S212P | E271V | | | | | | |
| N76D | S103A | V104I | S212P | Q236L | N243S | E271V | | | | | | |
| G20V | N62S | N76D | S103A | V104I | | | | | | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | |
| N76D | S103A | V104I | A232V | Q245R | | | | | | | | |
| S24T | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N252K | | | | | |
| V68A | N76D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | | |
| V68A | S103A | V104I | N140D | G159D | A232V | Q236H | Q245R | N252K | | | | |
| N43S | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N252K | | | | |
| N43K | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | |
| N43D | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N252K | | | | |
| V68A | S87G | S103A | V104I | G159D | A232V | Q236H | Q245R | N252K | R275S | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | | | | | |
| V68A | S103A | V104I | N116D | G159D | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | | | | | |
| R10C | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | V203E | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | K237E | Q245R | | | | | |
| V68A | N76D | I79N | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | |
| V68A | S103A | V104I | G159D | N183D | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | A174V | Q206L | A232V | Q236H | Q245R | | | | |
| V68A | S103A | V104I | G159D | S188C | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | A230T | A232V | Q236H | Q245R | | | | | |
| V68A | A98T | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | A215T | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248S | | | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | |

| V68A | N76D | S103A | V104I | G159D | P210R | A232V | Q236H | Q245R | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | | | | |
| N76D | S103A | V104I | A232V | Q236H | Q245R | L257V | | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | R275H | | | | |
| N76D | S103A | V104I | L257V | R275H | | | | | | | | |
| V68A | S103A | V104I | G159D | T224A | A232V | Q236H | Q245R | L257V | | | | |
| N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | | | | | |
| V68A | N76D | S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | | | | |
| V68A | N76D | S103A | V104I | G159D | G211R | A232V | Q236H | Q245R | | | | |
| V68A | N76D | S103A | V104I | G159D | G211V | A232V | Q236H | Q245R | | | | |
| Q12R | V68A | N76D | S103A | V104I | G159D | Y214L | A232V | Q236H | Q245R | | | |
| V68A | N76D | S103A | V104I | G159D | A215R | A232V | Q236H | Q245R | | | | |
| Q12R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | |
| G20R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | S259G | | | |
| V68A | S87R | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | T260V | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | N261G | | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | N261W | | | | |
| N76D | S103A | V104I | A232V | Q236H | S242P | Q245R | | | | | | |
| V68A | N76D | S103A | V104I | G159D | P210L | A232V | Q236H | Q245R | | | | |
| Q12R | A48V | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | |
| N76D | S103A | V104I | A232V | Q236H | Q245R | | | | | | | |
| N76D | S103A | V104I | G159D | Y192P | A232V | Q236H | Q245R | | | | | |
| N76D | S103A | V104I | V147I | G159D | A232V | Q236H | Q245R | N248S | K251R | | | |
| Q12R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | A272S | | | |
| V68A | N76D | S103A | V104I | G159D | N183K | Q206L | A232V | Q236H | Q245R | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | S256R | | | | |
| V68A | N76D | S103A | V104I | G159D | Q206R | A232V | Q236H | Q245R | | | | |
| K27R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | |
| V68A | N76D | S103A | V104I | N116T | G159D | R170S | N185S | A232V | Q236H | Q245R | | |
| G61E | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| N43D | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| V68A | S103A | V104I | G159D | S212P | A232V | Q236H | Q245R | N248D | N252K | | | |
| V68A | S103A | V104I | S99N | G159D | N184D | A232V | Q236H | Q245R | N248D | N252K | | |
| S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| V68A | S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | N248D | N252K | | | |
| V68A | S103A | V104I | Q109R | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G20R | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | Y209F | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | N261D | | | | |
| V68A | S103A | V104I | G159D | N185D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | P210R | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | P210T | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | P210S | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | N185D | P210L | A232V | Q236H | Q245R | N248D | N252K | | | |
| V68A | S103A | V104I | G159D | P210L | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | S212A | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | S212E | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | T213E | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | T213S | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| V68A | A103V | V104I | G159D | T213E | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | T213G | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | A215V | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | A215R | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | S216T | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | S216V | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | S216C | A232V | Q236H | Q245R | N248D | N252K | | | | |
| G20A | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | N173D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | K251V | N252K | | | | |
| V68A | S103A | V104I | G159D | Q206R | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252F | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252L | | | | | |
| P55S | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252F | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | T255V | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | S256N | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | S256E | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | S256R | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | T260R | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | L257R | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | G258D | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I8V | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | N269D | | | |
| V68A | S103A | V104I | N116S | G159D | A232V | Q236H | Q245R | N248D | N252K | T260E | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | N261R | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | N261D | | | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | A232V | Q236H | Q245R | N248D | N252K | | | | | | |
| S103A | V104I | G159D | A232S | Q236H | Q245R | N248D | N252K | | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236R | Q245R | N248D | N252K | | | | | |
| N18S | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245V | N248D | N252K | | | | | |
| V68A | N76D | S101T | S103A | V104I | G159D | T213R | N218S | A232V | Q236H | Q245R | T260A | | |
| V68A | S103A | V104I | G159D | A228V | A232V | Q236H | Q245R | N248D | N252K | | | | |
| T33S | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| V68A | N76D | E89D | S103A | V104I | G159D | P210L | T213R | A232V | Q236H | Q245R | T260A | | |
| G61E | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| S103A | V104I | G159D | V205I | P210I | A232V | Q236H | Q245R | | | | | | |
| G61E | V68A | S103A | V104I | S130A | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| G61E | V68A | S103A | V104I | A133S | Q137R | G159D | A232V | Q236H | Q245R | N248D | N252K | | |
| G61E | S103A | V104I | A133V | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248G | N252K | | | | | |
| V68A | S103A | V104I | G159D | N218S | A232V | Q236H | Q245R | N248D | N252K | | | | |
| G61E | V68A | S103A | V104I | G159D | S160V | A232V | Q236H | Q245R | N248D | N252K | | | |
| S3L | G61E | V68A | N76D | S103A | V104I | A232V | Q236H | Q245R | N248D | N252K | | | |
| G61E | V68A | S103A | V104I | G159D | S167F | A232V | Q236H | Q245R | N248D | N252K | | | |
| G97E | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| A98D | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S99E | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S101E | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S101G | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| G102A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S103A | V104I | S106E | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S103A | V104I | Q109E | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | N261R | | | | | |
| S103A | V104I | Q109R | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| N62D | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | | |
| S103A | V104I | G159D | N184D | A232V | Q236H | Q245R | N248D | N252K | | | | | |

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| S103A | V104I | G159D | S166D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| S103A | V104I | G159D | L217E | A232V | Q236H | Q245R | N248D | N252K | | | | |
| G20R | N62D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| N62D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | | |
| S103A | V104I | G159D | Q206R | L217E | A232V | Q236H | Q245R | N248D | N252K | | | |
| N62D | S103A | V104I | G159D | Q206R | A232V | Q236H | Q245R | N248D | N252K | | | |
| S103A | V104I | S130G | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| S103A | V104I | P131V | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| K27N | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| T38G | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| T38A | N76D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | | | |
| V68A | N76D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | E271G | | |
| V68A | N76D | S103A | V104I | G159D | Y209W | T213R | A232V | Q236H | Q245R | T260A | | |
| V68A | N76D | S103A | V104I | G159D | P210I | T213R | A232V | Q236H | Q245R | T260A | | |
| V68A | N76D | S103A | V104I | G159D | V205I | T213R | A232V | Q236H | Q245R | T260A | | |
| V68A | N76D | S103A | V104I | G159D | P210I | A232V | Q236H | Q245R | T260A | | | |
| V68A | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | | | | |
| N76D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | | | | |
| V68A | S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | P210I | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | A230V | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | L126F | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | V205I | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | G159D | P210L | A232V | Q236H | Q245R | | | | | |
| S103A | V104I | G159D | A230V | Q236H | Q245R | | | | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | T260A | | | | | |
| S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | | | |
| V68A | S103A | V104I | G159D | A174V | A232V | Q236H | Q245R | L257V | | | | |
| V68A | S103A | V104I | G159D | A194S | A232V | Q236H | Q245R | L257V | | | | |
| V68A | S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | L257V | | | | |
| S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | | | | | | |
| V68A | N76D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | N261W | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | N261W | | | | |
| S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | | | | | |
| S103A | V104I | G159D | P210I | A232V | Q236H | Q245R | N248D | N252K | | | | |
| S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | L257V | | | | | |

| V68A | N76D | S103A | V104I | G159D | P210L | T213R | A232V | Q236H | Q245R | T260A | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Q12R | S103A | V104I | G159D | Y209W | T213R | A232V | Q236H | Q245R | T260A | | | |
| S103A | V104I | Y209W | A232V | Q236H | Q245R | L257V | | | | | | |
| S103A | V104I | G159D | V205I | P210I | T213R | A232V | Q236H | Q245R | T260A | | | |
| S103A | V104I | G159D | V205I | Y209W | A232V | Q236H | Q245R | T260A | | | | |
| V68A | S103A | V104I | G159D | V205I | Y209W | P210I | A232V | Q236H | Q245R | | | |
| S103A | V104I | G159D | V205I | Y209W | P210I | A232V | Q236H | Q245R | L257V | | | |
| S103A | V104I | G159D | V205I | Y209W | A232V | Q236H | Q245R | L257V | | | | |
| V68A | S103A | V104I | G159D | V205I | Y209W | P210I | A232V | Q236H | Q245R | T260A | | |
| S103A | V104I | G159D | V205I | Y209W | P210I | A232V | Q236H | Q245R | | | | |
| S103A | V104I | G159D | Y209W | P210I | A232V | Q236H | Q245R | | | | | |
| S103A | V104I | G159D | V205I | P210I | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | S128L | G159D | A232V | Q236H | Q245R | | | | | |
| A48V | S103A | V104I | G159D | A230V | Q236H | Q245R | | | | | | |
| A48V | V68A | S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | | | | |
| A48V | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| A48V | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | N261W | | | |
| G102A | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | | |
| Q12R | G102A | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | |
| S101G | G102A | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | |
| A98L | G102A | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | |
| G102A | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | | |
| S103A | V104I | P131V | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| S103A | V104I | G159D | N184S | A232V | Q236H | Q245R | N248D | N252K | | | | |
| S103A | V104I | G159D | N184G | A232V | Q236H | Q245R | N248D | N252K | | | | |
| S103A | V104I | G159D | A232V | Q236H | V244T | Q245R | N248D | N252K | | | | |
| S103A | V104I | G159D | A232V | Q236H | V244A | Q245R | N248D | N252K | | | | |
| N62D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | S256R | | |
| Q12R | N62D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| S101G | S103A | V104I | G159D | N185D | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | G159D | Q206E | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | G159D | T213Q | A232V | Q236H | Q245R | N248D | N252K | | | |
| A98L | G102A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | G102A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| A98L | G102A | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | |
| A98L | G102A | S103A | V104I | G159D | S212G | A232V | Q236H | N248D | N252K | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N62D | S103A | V104I | Q109R | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| N62D | S103A | V104I | G159D | S212G | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| N62D | S101G | S103A | V104I | G159D | S212G | T213R | A232V | Q236H | Q245R | N248D | N252K | |
| S103A | V104I | G159D | A232V | Q245R | N248D | N252K | | | | | | |
| S103A | V104I | G159D | A230V | Q245R | | | | | | | | |
| N62D | S103A | V104I | S130G | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| S101G | S103A | V104I | S130G | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | S128G | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | S128L | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| N62D | S101G | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| N62D | S103A | V104I | S128G | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| N62D | S103A | V104I | S128L | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| S101G | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | T260A | | | |
| S101G | S103A | V104I | P131V | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| A98V | S101G | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| S99G | S101G | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | G159D | P210I | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | G159D | V205I | A232V | Q236H | Q245R | N248D | N252K | | | |
| S101G | S103A | V104I | G159D | A230V | Q236H | Q245R | | | | | | |
| S101G | S103A | V104I | G159D | A194P | A232V | Q236H | Q245R | N248D | N252K | | | |
| N76D | S101G | S103A | V104I | G159D | A194P | A232V | Q236H | Q245R | N248D | N252K | | |
| S101G | S103A | V104I | G159D | A230V | A232V | Q236H | Q245R | N248D | N252K | | | |
| N62D | S103A | V104I | G159D | N185D | Q206E | T213R | A232V | Q236H | Q245R | N248D | N252K | E271Q |

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table VI except for the following substitution set of Table VIII:

Table VIII

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 68 | 76 | 103 | 104 | 116 | 159 | 170 | 185 | 232 | 236 | 245 |

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table IX:

Table IX

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 252 | | | | |
| 68 | 76 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 260 | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | | | | | |
| 68 | 103 | 104 | 140 | 159 | 232 | 236 | 245 | 252 | | | |
| 43 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 252 | | | |
| 43 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | | | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 257 | | | | |
| 68 | 76 | 103 | 104 | 159 | 210 | 232 | 236 | 245 | | | |
| 68 | 103 | 104 | 159 | 224 | 232 | 236 | 245 | 257 | | | |
| 76 | 103 | 104 | 159 | 232 | 236 | 245 | 257 | | | | |
| 68 | 76 | 103 | 104 | 159 | 211 | 232 | 236 | 245 | | | |
| 12 | 68 | 76 | 103 | 104 | 159 | 214 | 232 | 236 | 245 | | |
| 68 | 76 | 103 | 104 | 159 | 215 | 232 | 236 | 245 | | | |
| 12 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | |
| 20 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 259 | | |
| 68 | 76 | 87 | 103 | 104 | 159 | 232 | 236 | 245 | 260 | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 261 | | | |
| 12 | 48 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | |
| 76 | 103 | 104 | 159 | 192 | 232 | 236 | 245 | | | | |
| 76 | 103 | 104 | 147 | 159 | 232 | 236 | 245 | 248 | 251 | | |
| 12 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 272 | | |
| 68 | 76 | 103 | 104 | 159 | 183 | 206 | 232 | 236 | 245 | | |
| 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | 256 | | | |
| 68 | 76 | 103 | 104 | 159 | 206 | 232 | 236 | 245 | | | |
| 27 | 68 | 76 | 103 | 104 | 159 | 232 | 236 | 245 | | | |
| 68 | 103 | 104 | 159 | 212 | 232 | 236 | 245 | 248 | 252 | | |
| 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | | | |
| 68 | 103 | 104 | 159 | 209 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 109 | 159 | 232 | 236 | 245 | 248 | 252 | | |
| 20 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 68 | 103 | 104 | 159 | 209 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 159 | 210 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 159 | 212 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 159 | 213 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 213 | 232 | 236 | 245 | 248 | 252 | | | |
| 68 | 103 | 104 | 159 | 215 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 159 | 216 | 232 | 236 | 245 | 248 | 252 | | |
| 20 | 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 255 | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 256 | | |
| 68 | 103 | 104 | 159 | 232 | 236 | 245 | 248 | 252 | 260 | | |
| 68 | 103 | 104 | 159 | 228 | 232 | 236 | 245 | 248 | 252 | | |
| 68 | 76 | 89 | 103 | 104 | 159 | 210 | 213 | 232 | 236 | 245 | 260 |
| 68 | 103 | 104 | 159 | 218 | 232 | 236 | 245 | 248 | 252 | | |

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table X:

Table X

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| V68A | S103A | V104I | G159D | A228V | A232V | Q236H | Q245R | N248D | N252K | | |
| V68A | S103A | V104I | G159D | N218S | A232V | Q236H | Q245R | N248D | N252K | | |
| G20R | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | |
| V68A | N76D | E89D | S103A | V104I | G159D | P210L | T213R | A232V | Q236H | Q245R | T260A |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | S256R | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | T260R | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | T255V | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | S256N | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252L | | | |
| V68A | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | N248D | N252K | | |
| V68A | S103A | V104I | G159D | A215V | A232V | Q236H | Q245R | N248D | N252K | | |
| V68A | S103A | V104I | G159D | A215R | A232V | Q236H | Q245R | N248D | N252K | | |
| V68A | S103A | V104I | G159D | S216T | A232V | Q236H | Q245R | N248D | N252K | | |
| V68A | S103A | V104I | G159D | S216V | A232V | Q236H | Q245R | N248D | N252K | | |
| V68A | S103A | V104I | T213S | A232V | Q236H | Q245R | N248D | N252K | | | |

| V68A | S103A | V104I | G159D | P210L | A232V | Q236H | Q245R | N248D | N252K | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| V68A | S103A | V104I | G159D | S212C | A232V | Q236H | Q245R | N248D | N252K | | |
| V68A | S103A | V104I | G159D | S212G | A232V | Q236H | Q245R | N248D | N252K | | |
| S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | | |
| V68A | S103A | V104I | G159D | Y209W | A232V | Q236H | Q245R | N248D | N252K | | |
| V68A | S103A | V104I | Q109R | G159D | A232V | Q236H | Q245R | N248D | N252K | | |
| G20R | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | |
| V68A | S103A | V104I | G159D | V209F | A232V | Q236H | Q245R | N248D | N252K | | |
| N76D | S103A | V104I | G159D | Y192F | A232V | Q236H | Q245R | | | | |
| N76D | S103A | V104I | V147I | G159D | A232V | Q236H | Q245R | N248S | K251R | | |
| Q12R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | A272S | | |
| V68A | N76D | S103A | V104I | G159D | N183K | Q206L | A232V | Q236H | Q245R | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | S256R | | | |
| V68A | N76D | S103A | V104I | G159D | Q206R | A232V | Q236H | Q245R | | | |
| K27R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | |
| Q12R | A48V | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | |
| V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | N261W | | | |
| V68A | N76D | S103A | V104I | G159D | G211R | A232V | Q236H | Q245R | | | |
| V68A | N76D | S103A | V104I | G159D | G211V | A232V | Q236H | Q245R | | | |
| Q12R | V68A | N76D | S103A | V104I | G159D | Y214L | A232V | Q236H | Q245R | | |
| V68A | N76D | S103A | V104I | G159D | A215R | A232V | Q236H | Q245R | | | |
| Q12R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | | | |
| G20R | V68A | N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | S259G | | |
| V68A | N76D | S87R | S103A | V104I | G159D | A232V | Q236H | Q245R | T260V | | |
| N76D | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | | | | |
| V68A | N76D | S103A | V104I | G159D | T213R | A232V | Q236H | Q245R | T260A | | |
| V68A | N76D | S103A | V104I | G159D | P210R | A232V | Q236H | Q245R | | | |
| V68A | S103A | V104I | G159D | S212P | A232V | Q236H | Q245R | N248D | N252K | | |
| V68A | S103A | V104I | G159D | T224A | A232V | Q236H | Q245R | L257V | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N252S | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N252K | | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N248D | N252K | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | | |
| V68A | S103A | V104I | N140D | G159D | A232V | Q236H | Q245R | N252K | | | |
| N43S | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N252K | | | |
| N43K | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N43D | V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | N252K | | | |
| V68A | S103A | V104I | G159D | A232V | Q236H | Q245R | L257V | | | | |

A highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

12/102/103/104/159/212/232/236/245/248/252;
62/103/104/130/159/213/232/236/245/248/252;
62/103/104/109/159/213/232/236/245/248/252;
62/101/103/104/159/212/213/232/236/245/248/252;
68/103/104/159/232/236/245/248/252/270;
68/103/104/159/185/232/236/245/248/252;
68/103/104/159/185/210/232/236/245/248/252;
68/103/104/159/230/232/236/245;
68/103/104/232/236/245/248/257/275;
68/103/104/159/232/236/245/248/252;
68/76/103/104/159/232/236/245;
68/103/104/159/232/236/245;
68/76/103/104/159/211/232/236/245;
68/103/104/159/210/232/236/245;
68/76/103/104/159/213/232/236/245/260;
68/103/104/159/183/232/236/245/248/252;
68/103/104/159/213/232/236/245;
76/103/104/159/213/232/236/245/260;
97/103/104/159/232/236/245/248/252;
98/102/103/104/159/212/232/236/245/248/252;
102/103/104/159/232/236/245/248/252;
103/104/159/248/252/270;
103/104/159/205/209/232/236/245/257
103/104/159/205/209/210/232/236/245/257;
103/104/159/217/232/236/245/248/252;
103/104/131/159/232/236/245/248/252;
103/104/159/232/236/245/257.

61/68/103/104/159/232/236/245/248/252;
62/103/104/159/213/232/236/245/248/252;
62/103/104/159/232/236/245/248/252;
68/103/104/159/210/232/236/245/248/252;
68/103/104/159/213/232/236/245/248/252;
68/76/103/104/159/209/232/236/245;
68/103/104/213/232/236/245/248/252;
68/103/104/159/209/232/236/245;
68/103/104/159/232/236/245/252;
68/103/104/159/232/236/245/257;
68/76/103/104/159/215/232/236/245;
68/103/104/159/213/232/236/245/260;
68/76/103/104/159/210/232/236/245/260;
68/103/104/232/236/245/257/275;
76/103/104/159/232/236/245;
76/103/104/131/159/232/236/245/248/252;
98/103/104/159/232/236/245/248/252;
101/103/104/159/232/236/245/248/252;
103/104/159/232/236/245;
103/104/159/232/236/245/248/252;
103/104/159/232/245/248/252;
103/104/159/213/232/236/245/248/252;
103/104/130/159/232/236/245/248/252;
103/104/159/205/209/232/236/245; and

A more highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

12R/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K;
61E/68A/103A/104I/159D/232V/236H/245R/248D/252K;
62D/103A/104I/109R/159D/213R/232V/236H/245R/248D/252K;
62D/103A/104I/159D/213R/232V/236H/245R/248D/252K;
62D/103A/104I/159D/232V/236H/245R/248D/252K;
62D/103A/104I/130G/159D/213R/232V/236H/245R/248D/252K;
62D/101G/103A/104I/159D/212G/213R/232V/236H/245R/248D/252K;
68A/76D/103A/104I/159D/213R/232V/236H/245R/260A;
68A/76D/103A/104I/159D/210I/232V/236H/245R/260A;
68A/103A/104I/159D/183D/232V/236H/245R/248D/252K;
68A/103A/104I/159D/209W/232V/236H/245R;
68A/76D/103A/104I/159D/211R/232V/236H/245R;
68A/76D/103A/104I/159D/215R/232V/236H/245R;
68A/103A/104I/159D/213R/232V/236H/245R/260A;
68A/76D/103A/104I/159D/232V/236H/245R;
68A/103A/104I/159D/232V/236H/245R/252K;
68A/103A/104I/159D/232V/236H/245R;
68A/103A/104I/159D/232V/236H/245R/257V;
68A/103A/104I/159D/185D/232V/236H/245R/248D/252K;
68A/103A/104I/159D/210L/232V/236H/245R/248D/252K;
68A/103A/104I/159D/185D/210L/232V/236H/245R/248D/252K;
68A/103A/104I/159D/213E/232V/236H/245R/248D/252K;
68A/103A/104I/159D/230V/232V/236H/245R;
68A/76D/103A/104I/159D/209W/232V/236H/245R;
68A/103A/104I/232V/236H/245R/248D/257V/275H;
68A/103A/104I/232V/236H/245R/257V/275H;
68A/103A/104I/213E/232V/236H/245R/248D/252K;
68A/103A/104I/159D/232V/236H/245R/248D/252K;
68A/103A/104I/159D/210I/232V/236H/245R;
68A/103A/104I/159D/210L/232V/236H/245R;
68A/103A/104I/159D/213G/232V/236H/245R;
68A/103A/104I/159D/232V/236H/245R/248D/252K/270A;
76D/103A/104I/159D/232V/236H/245R;
76D/103A/104I/131V/159D/232V/236H/245R/248D/252K;
76D/103A/104I/159D/213R/232V/236H/245R/260A;
97E/103A/104I/159D/232V/236H/245R/248D/252K;
98L/103A/104I/159D/232V/236H/245R/248D/252K;

98L/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K;
101G/103A/104I/159D/232V/236H/245R/248D/252K;
102A/103A/104I/159D/232V/236H/245R/248D/252K;
103A/104I/159D/232V/236H/245R/248D/252K;
103A/104I/159D/213R/232V/236H/245R/248D/252K;
103A/104I/130G/159D/232V/236H/245R/248D/252K;
103A/104I/159D/217E/232V/236H/245R/248D/252K;
103A/104I/159D/248D/252K/270V;
103A/104I/159D/232V/236H/245R;
103A/104I/159D/205I/209W/232V/236H/245R;
103A/104I/159D/232V/236H/245R/257V;
103A/104I/159D/205I/209W/232V/236H/245R/257V;
103A/104I/131V/159D/232V/236H/245R/248D/252K;
103A/104I/159D/205I/209W/210I/232V/236H/245R/257V; and
103A/104I/159D/232V/245R/248D/252K.

Recombinant Proteases/Recombinant Subtilisins - A "recombinant protease" or "recombinant subtilisin" refers to a protease or subtilisin in which the DNA sequence encoding the naturally-occurring protease or subtilisin, respectively, is modified to produce a mutant DNA sequence which encodes the substitution, insertion or deletion of one or more amino acids in the protease or subtilisin amino acid sequence. Suitable modification methods are disclosed herein, and in U.S. Patent Nos. RE 34,606, 5,204,015 and 5,185,258.

Non-Human Proteases/Non-Human Subtilisins - "Non-human proteases" or "non-human subtilisins" and the DNA encoding them may be obtained from many procaryotic and eucaryotic organisms. Suitable examples of procaryotic organisms include gram negative organisms such as *E. coli* or *Pseudomonas* and gram positive bacteria such as *Micrococcus* or *Bacillus*. Examples of eucaryotic organisms from which carbonyl hydrolase and their genes may be obtained include yeast such as *Saccharomyces cerevisiae*, fungi such as *Aspergillus* sp. and non-human mammalian sources such as, for example, *bovine* sp. from which the gene encoding the protease chymosin or subtilisin chymosin can be obtained. A series of proteases and/or subtilisins can be obtained from various related species which have amino acid sequences which are not entirely homologous between the members of that series but which nevertheless exhibit the same or similar type of biological activity. Thus, non-human protease or non-human subtilisin as used herein have a functional definition which refers to proteases or subtilisins, respectively, which are associated, directly or indirectly, with procaryotic and eucaryotic sources.

Variant DNA Sequences - Variant DNA sequences encoding such protease or subtilisin variants are derived from a precursor DNA sequence which encodes a naturally-occurring or recombinant precursor enzyme.

In a preferred embodiment of the present invention, the variant DNA sequences are derived by modifying the precursor DNA sequence to encode the substitution, insertion or deletion of one or more specific amino acid residues encoded by the precursor DNA sequence corresponding to positions 103 in combination with one or more of the following positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of *Bacillus amyloliquefaciens* subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a substitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of *Bacillus amyloliquefaciens* subtilisin. More preferably, these variant DNA sequences encode the protease variants described herein.

In another preferred embodiment, these variant DNA sequences encode the substitution, insertion or deletion of one or more of the amino acid residues corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin. More preferably, these variant DNA sequences encode the protease variants described herein.

Although the amino acid residues identified for modification herein are identified according to the numbering applicable to *B. amyloliquefaciens* (which has become the conventional method for identifying residue positions in all subtilisins), the preferred precursor DNA sequences useful for the present invention is the DNA sequence of *Bacillus lentus* as shown in Fig. 3.

These recombinant DNA sequences encode protease variants having a novel amino acid sequence and, in general, at least one property which is substantially different from the same property of the enzyme encoded by the precursor protease DNA sequence. Such properties include proteolytic activity, substrate specificity, stability, altered pH profile and/or enhanced performance characteristics.

Specific substitutions corresponding to positions 103 in combination with one or more of the following positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27,

33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of *Bacillus amyloliquefaciens* subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a substitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 wherein the numbered positions correspond to the naturally-occurring subtilisin from *Bacillus amyloliquefaciens* or to equivalent amino acid residues in other carbonyl hydrolases or subtilisins (such as *Bacillus lentus* subtilisin) are described herein. Further, specific substitutions corresponding to one or more of the following positions 62, 212, 230, 232, 252 and 257 wherein the numbered positions correspond to the naturally-occurring subtilisin from *Bacillus amyloliquefaciens* or to equivalent amino acid residues in other carbonyl hydrolases or subtilisins (such as *Bacillus lentus* subtilisin) are described herein. These amino acid position numbers refer to those assigned to the mature *Bacillus amyloliquefaciens* subtilisin sequence presented in Fig. 1. The present invention, however, is not limited to the use of mutation of this particular subtilisin but extends to precursor proteases containing amino acid residues at positions which are "equivalent" to the particular identified residues in *Bacillus amyloliquefaciens* subtilisin. In a preferred embodiment of the present invention, the precursor protease is *Bacillus lentus* subtilisin and the substitutions, deletions or insertions are made at the equivalent amino acid residue in *B. lentus* corresponding to those listed above.

A residue (amino acid) of a precursor protease is equivalent to a residue of *Bacillus amyloliquefaciens* subtilisin if it is either homologous (i.e., corresponding in position in either primary or tertiary structure) or analogous to a specific residue or portion of that residue in *Bacillus amyloliquefaciens* subtilisin (i.e., having the same or similar functional capacity to combine, react or interact chemically).

In order to establish homology to primary structure, the amino acid sequence of a precursor protease is directly compared to the *Bacillus amyloliquefaciens* subtilisin primary sequence and particularly to a set of residues known to be invariant in subtilisins for which sequence is known. For example, Fig. 2 herein shows the conserved residues as between *B. amyloliquefaciens* subtilisin and *B. lentus* subtilisin. After aligning the conserved residues, allowing for necessary insertions and deletions in order to maintain alignment (i.e.,

avoiding the elimination of conserved residues through arbitrary deletion and insertion), the residues equivalent to particular amino acids in the primary sequence of *Bacillus amyloliquefaciens* subtilisin are defined. Alignment of conserved residues preferably should conserve 100% of such residues. However, alignment of greater than 75% or as little as 50% of conserved residues is also adequate to define equivalent residues. Conservation of the catalytic triad, Asp32/His64/Ser221 should be maintained.

For example, in Fig. 3 the amino acid sequence of subtilisin from *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus licheniformis (carlsbergensis)* and *Bacillus lentus* are aligned to provide the maximum amount of homology between amino acid sequences. A comparison of these sequences shows that there are a number of conserved residues contained in each sequence. These conserved residues (as between BPN' and *B. lentus*) are identified in Fig. 2.

These conserved residues, thus, may be used to define the corresponding equivalent amino acid residues of *Bacillus lentus* (PCT Publication No. WO89/06279 published July 13, 1989), the preferred protease precursor enzyme herein, or the subtilisin referred to as PB92 (EP 0 328 299), which is highly homologous to the preferred *Bacillus lentus* subtilisin. The amino acid sequences of certain of these subtilisins are aligned in Figs. 3A and 3B with the sequence of *Bacillus amyloliquefaciens* subtilisin to produce the maximum homology of conserved residues. As can be seen, there are a number of deletion in the sequence of *Bacillus lentus* as compared to *Bacillus amyloliquefaciens* subtilisin. Thus, for example, the equivalent amino acid for Val165 in *Bacillus amyloliquefaciens* subtilisin in the other subtilisins is isoleucine for *B. lentus and B. licheniformis*. Thus, for example, the amino acid at position +76 is asparagine (N) in both *B. amyloliquefaciens* and *B. lentus subtilisins*. In the protease variants of the invention, however, the amino acid equivalent to +76 in *Bacillus amyloliquefaciens* subtilisin is substituted with aspartate (D). The abbreviations and one letter codes for all amino acids in the present invention conform to the Patentin User Manual (GenBank, Mountain View, CA) 1990, p. 101.

"Equivalent residues" may also be defined by determining homology at the level of tertiary structure for a precursor protease whose tertiary structure has been determined by x-ray crystallography. Equivalent residues are defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of the precursor protease and *Bacillus amyloliquefaciens* subtilisin (N on N, CA on CA, C on C and O on O) are within 0.13nm and preferably 0.1nm after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the protease in question to the *Bacillus amyloliquefaciens* subtilisin. The best model is the crystallographic model

giving the lowest R factor for experimental diffraction data at the highest resolution available.

$$R\ factor = \frac{\Sigma_h ||Fo(h)| - |Fc(h)||}{\Sigma_h |Fo(h)|}$$

Equivalent residues which are functionally analogues to a specific residue of *Bacillus amyloliquefaciens* subtilisin are defined as those amino acids of the precursor protease which may adopt a conformation such that they either alter, modify or contribute to protein structure, substrate binding or catalysis in a manner defined and attributed to a specific residue of the *Bacillus amyloliquefaciens* subtilisin. Further, they are those residues of the precursor protease (for which a tertiary structure has been obtained by x-ray crystallography) which occupy an analogous position to the extent that, although the main chain atoms of the given residue may not satisfy the criteria of equivalence on the basis of occupying a homologous position, the atomic coordinates of at least two fo the side chain atoms of the residue lie with 0.13nm of the corresponding side chain atoms of *Bacillus amyloliquefaciens* subtilisin. The coordinates of the three dimensional structure of *Bacillus amyloliquefaciens* subtilisin are set forth in EPO Publication No. 0 251 446 (equivalent to US Patent 5,182,204, the disclosure of which is incorporated herein by reference) and can be used as outlined above to determine equivalent residues on the level of tertiary structure.

Some of the residues identified for substitution, insertion or deletion are conserved residues whereas others are not. In the case of residues which are not conserved, the replacement of one or more amino acids is limited to substitutions which produce a variant which has an amino acid sequence that does not correspond to one found in nature. In the case of conserved residues, such replacements should not result in natural-occurring sequence. The protease variants of the present invention include the mature forms of protease variants, as well as the pro- and pre-pro-forms of such protease variants. The prepro-forms are the preferred construction since this facilitates the expression, secretion and maturation of the protease variants.

"Prosequence" refers to a sequence of amino acids bound to the N-terminal portion of the mature form of a protease which when removed results in the appearance of the "mature" form of the protease. Many proteolytic enzymes are found in nature as translational proenzyme products and, in the absence of post-translational processing, are expressed in this fashion. A preferred prosequence for producing protease variants is the putative prosequence of *Bacillus amyloliquefaciens* subtilisin, although other protease prosequences may be used.

A "signal sequence" or "presequence" refers to any sequence of amino acids bound to the N'terminal portion of a protease or to the N-terminal portion of a proprotease which may participate in the secretion of the mature or pro forms of the protease. This definition

of signal sequence is a functional one, meant to include all those amino sequences encoded by the N-terminal portion of the protease gene which participate in the effectuation of the secretion of protease under native conditions. The present invention utilizes such sequences to effect the secretion of the protease variants as defined here. One possible signal sequence comprises the first seven amino acid residues of the signal sequence from *Bacillus subtilis* subtilisin fused to the remainder of the signal sequence of the subtilisin from *Bacillus lentus,(ATCC 21536)*.

A "prepro" form of a protease variant consists of the mature form of the protease having a prosequence operably linked to the amino terminus of the protease and a "pre" or "signal" sequence operably linked to the amino terminus of the prosequence.

"Expression vector" refers to a DNA construct containing a DNA sequence which is operably linked to a suitable control sequence capable of effecting the expression of said DNA in a suitable host. Such control sequences include a promoter to effect transcription, an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome binding sites and sequences which control termination of transcription and translation. The vector may be a plasmid, a phage particle, or simply a potential genomic insert. Once transformed into a suitable host, the vector may replicate and function independently or the host genome, or may, in some instances, integrate into the genome itself. In the present specification, "plasmid" and "vector" are sometimes used interchangeably as the plasmid is the most commonly used form of vector at present. However, the invention is intended to include such other forms of expression vectors which serve equivalent functions and which are, or become, known in the art.

The "host cells" used in the present invention generally are procaryotic or eucaryotic hosts which preferably have been manipulated by the methods disclosed in US Patent RE 34,606 to render them incapable of secreting enzymatically active endoprotease. A preferred host cell for expressing protease is the *Bacillus* strain BG2036 which is deficient in enzymatically active neutral protease and alkaline protease (subtilisin). The construction of strain BG2036 is described in detail in US Patent 5,264,366. Other host cells for expressing protease include *Bacillus subtilis* 168 (also described in US Patent RE 34,606 and US Patent 5,264,366, the disclosure of which are incorporated herein by reference), as well as any suitable *Bacillus* strain such as *B. licheniformis, B. lentus*,etc.).

Host cells are transformed or transfected with vectors constructed using recombinant DNA techniques. Such transformed host cells are capable of either replicating vectors encoding the protease variants or expressing the desired protease variant. In the case of vectors which encode the pre- or prepro-form of the protease variant, such variants, when expressed, are typically secreted from the host cell in to the host cell medium.

"Operably linked, "when describing the relationship between two DNA regions, simply means that they are functionally related to each other. For example, a prosequence is operably linked to a peptide if it functions as a signal sequence, participating in the secretion of the mature form of the protein most probably involving cleavage of the signal sequence. A promoter is operably linked to a coding sequence if it controls the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation.

The genes encoding the naturally-occurring precursor protease may be obtained in accord with the general methods known to those skilled in the art. The methods generally comprise synthesizing labeled probes having putative sequences encoding regions of the protease of interest, preparing genomic libraries from organisms expressing the protease, and screening the libraries for the gene of interest by hybridization to the probes. Positively hybridizing clones are then mapped and sequenced.

The cloned protease is then used to transform a host cell in order to express the protease. The protease gene is then ligated into a high copy number plasmid. This plasmid replicates in hosts in the sense that it contains the well-known elements necessary for plasmid replication: a promote operably linked to the gene in question (which may be supplied as the gene's own homologous promoter if it is recognized, i.e. transcribed by the host), a transcription termination and polyadenylation region (necessary for stability of the mRNA transcribed by the host from the protease gene in certain eucaryotic host cells) which is exogenous or is supplied by the endogenous terminator region of the protease gene and, desirably, a selection gene such as an antibiotic resistance gene that enables continuous cultural maintenance of plasmid-infected host cells by growth in antibiotic-containing media. High copy number plasmids also contain an origin of replication for the host, thereby enabling large numbers of plasmids to be generated in the cytoplasm without chromosomal limitation. However, it is within the scope herein to integrate multiple copies of the protease gene into host genome. This is facilitated by procaryotic and eucaryotic organisms which are particularly susceptible to homologous recombination. The gene can be a natural *B. lentus* gene. Alternatively, a synthetic gene encoding a naturally-occurring or mutant precursor protease may be produced. In such an approach, the DNA and/or amino acid sequence of the precursor protease is determined. Multiple, overlapping synthetic single-stranded DNA fragments are thereafter synthesized, which upon hybridization and ligation produce a synthetic DNA encoding the precursor protease. An example of synthetic gene construction is set forth in Example 3 of US Patent 5,204,105, the disclosure of which is incorporated herein by reference.

Once the naturally-occurring or synthetic precursor protease gene has been cloned, a number of modifications are undertaken to enhance the use of the gene beyond synthesis

of the naturally-occurring precursor protease. Such modifications include the production of recombinant proteases as disclosed in US Patent RE 34,606 and EPO Publication No. 0 251 446 and the production of protease variants described herein.

The following cassette mutagenesis method may be used to facilitate the construction of the proteases variants of the present invention, although other methods may be used. First, the naturally-occurring gene encoding the protease is obtained and sequenced in whole or in part. Then the sequence is scanned for a point at which it is desired to make a mutation (deletion, insertion or substitution) of one or more amino acids in the encoded enzyme. The sequences flanking this point are evaluated for the presence of restriction sites for replacing a short segment of the gene with an oligonucleotide pool which, when expressed will encode various mutants. Such restriction sites are preferably unique sites within the protease gene so as to facilitate the replacement of the gene segment. However, any convenient restriction site which is not overly redundant in the protease gene may be used, provided the gene fragments generated by restriction digestion can be reassembled in proper sequence. If restriction sites are not present at locations within a convenient distance from the selected point (from 10 to 15 nucleotides), such sites are generated by substituting nucleotides in the gene in such fashion that neither the reading frame nor the amino acids encoded are changed in the final construction. Mutation of the gene in order to change its sequence to conform to the desired sequence is accomplished by M13 primer extension in accord with generally known methods. The task of locating suitable flanking regions and evaluating the needed changes to arrive at two convenient restriction site sequences is made routine by the redundancy of the genetic code, a restriction enzyme map of the gene and the large number of different restriction enzymes. Note that if a convenient flanking restriction site if available, the above method need be used only in connection with the flanking region which does not contain a site.

Once the naturally-occurring DNA or synthetic DNA is cloned, the restriction sites flanking the positions to be mutated are digested with the cognate restriction enzymes and a plurality of end termini-complementary oligonucleotide cassettes are ligated into the gene. The mutagenesis is simplified by this method because all of the oligonucleotides can be synthesized so as to have the same restriction sites, and no synthetic linkers are necessary to create the restriction sites. As used herein, proteolytic activity is defined as the rate of hydrolysis of peptide bonds per milligram of active enzyme. Many well known procedures exist for measuring proteolytic activity (K. M. Kalisz, "Microbial Proteinases," Advances in Biochemical Engineering/Biotechnology, A. Fiechter ed., 1988). In addition to or as an alternative to modified proteolytic activity, the variant enzymes of the present invention may have other modified properties such as $K_m$, $k_{cat}$, $k_{cat}/K_m$ ratio and/or modified substrate specificity and/or modified pH activity profile. These enzymes can be tailored for the

particular substrate which is anticipated to be present, for example, in the preparation of peptides or for hydrolytic processes such as laundry uses.

In one aspect of the invention, the objective is to secure a variant protease having altered proteolytic activity as compared to the precursor protease, since increasing such activity (numerically larger) enables the use of the enzyme to more efficiently act on a target substrate. Also of interest are variant enzymes having altered thermal stability and/or altered substrate specificity as compared to the precursor. In some instances, lower proteolytic activity may be desirable, for example a decrease in proteolytic activity would be useful where the synthetic activity of the proteases is desired (as for synthesizing peptides). One may wish to decrease this proteolytic activity, which is capable of destroying the product of such synthesis. Conversely, in some instances it may be desirable to increase the proteolytic activity of the variant enzyme versus its precursor. Additionally, increases or decreases (alteration) of the stability of the variant, whether alkaline or thermal stability, may be desirable. Increases or decreases in $k_{cat}$, $K_m$ or $K_{cat}/K_m$ are specific to the substrate used to determine these kinetic parameters.

In another aspect of the invention, it has been determined that substitutions at positions corresponding to 103 in combination with one or more of the following positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of *Bacillus amyloliquefaciens* subtilisin are important in modulating overall stability and/or proteolytic activity of the enzyme.

In a further aspect of the invention, it has been determined that substitutions at one or more of the following positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin are also important in modulating overall stability and/or proteolytic activity of the enzyme.

These substitutions are preferably made in *Bacillus lentus* (recombinant or native-type) subtilisin, although the substitutions may be made in any *Bacillus* protease.

Based on the screening results obtained with the variant proteases, the noted mutations in *Bacillus amyloliquefaciens* subtilisin are important to the proteolytic activity, performance and/or stability of these enzymes and the cleaning or wash performance of such variant enzymes.

Methods and procedures for making the enzymes used in the detergent and bleaching compositions of the present invention are known and are disclosed in PCT Publication No. WO 95/10615.

The enzymes of the present invention have trypsin-like specificity. That is, the enzymes of the present invention hydrolyze proteins by preferentially cleaving the peptide bonds of charged amino acid residues, more specifically residues such as arginine and lysine, rather than preferentially cleaving the peptide bonds of hydrophobic amino acid residues, more specifically phenylalanine, tryptophan and tyrosine. Enzymes having the latter profile have a chymotrypsin-like specificity. Substrate specificity as discussed above is illustrated by the action of the enzyme on two synthetic substrates. Protease's having trypsin-like specificity hydrolyze the synthetic substrate bVGR-pNA preferentially over the synthetic substrate sucAAPF-pNA. Chymotrypsin-like protease enzymes, in contrast, hydrolyze the latter much faster than the former. For the purposes of the present invention the following procedure was employed to define the trypsin-like specificity of the protease enzymes of the present invention:

A fixed amount of a glycine buffer at a pH of 10 and a temperature of 25 °C is added to a standard 10 ml test tube. 0.5 ppm of the active enzyme to be tested is added to the test tube. Approximately, 1.25 mg of the synthetic substrate per mL of buffer solution is added to the test tube. The mixture is allowed to incubate for 15 minutes at 25 °C. Upon completion of the incubation period, an enzyme inhibitor, PMSF, is added to the mixture at a level of 0.5 mg per mL of buffer solution. The absorbency or OD value of the mixture is read at a 410 nm wavelength. The absorbence then indicates the activity of the enzyme on the synthetic substrate. The greater the absorbence, the higher the level of activity against that substrate.

To then determine the specificity of an individual enzyme, the absorbence on the two synthetic substrate proteins may be converted into a specificity ratio. For the purposes of the present invention, the ratio is determined by the formula specificity of:

[activity on sAAPF-pNA]/[activity on bVGR-pNA]

An enzyme having a ratio of less than about 10, more preferably less than about 5 and most preferably less than about 2.5 may then be considered to demonstrate trypsin-like activity.

Such variants generally have at least one property which is different from the same property of the protease precursor from which the amino acid sequence of the variant is derived.

One aspect of the invention are compositions, such as detergent and bleaching compositions, for the treatment of textiles, dishware, tableware, kitchenware, cookware, and other hard surface substrates that include one or more of the variant proteases of the present invention. Protease-containing compositions can be used to treat for example: silk

or wool, as well as other types of fabrics, as described in publications such as RD 216,034, EP 134,267, US 4,533,359, and EP 344,259; and dishware, tableware, kitchenware, cookware, and other hard surface substrates as described in publications such as in US 5,478,742, US 5,346,822, US 5,679,630, and US 5,677,272.

II. Bleaching Agents - The bleaching compositions herein contain a bleaching agent, which preferably comprises from about 0.5 to about 20 wt.% of the composition. The bleaching agent is either a substantially insoluble, preferably solid, organic peroxyacid, or a bleaching system comprising a bleach activator and a peroxygen bleaching compound capable of yielding hydrogen peroxide, or a combination of both. The peracid which is in the composition, or which is formed by the combination of activator and peroxygen compound, preferably has a corresponding carboxylic acid that has a Hydrophilic-Lipophilic Balance ("H.L.B.") value which ranges from about 3 to about 6.5. Therefore, a method that can be used to characterize the preferred peroxyacids (from activators or as preformed peroxyacids) which are useful in the present invention is the "H.L.B. Scale" such as that described in Davies, J.T., Proc 2nd Internat. Congr. Surface Activity 1, 426, Butterworths, London (1957), incorporated herein by reference. Such an H.L.B. Scale (Hydrophilic-Lipophilic Balance) has been used in the study of surface-active agents (surfactants) as a means to relate the distribution of a surface-active agent between a hydrophilic (water-like) and a lipophilic (oil-like) phase. In this manner, H.L.B. values can be used as an indication of the lipophilic (hydrophobic) character of the active bleaching species in the wash (i.e., the ability of the peroxyacid to partition out of the wash liquor and concentrate at the soil/fabric interface).

Set forth hereinafter in Table A are H.L.B. values which have been calculated for selected peroxyacids (as the corresponding carboxylic acids). The equation used to calculate the H.L.B. values can be set forth as:

*HLB = Sum (Hydrophilic Group Numbers) - Sum (Hydrophobic Group Numbers) + 7.*

The values for the Hydrophilic Group Numbers are [-C(O)OH & -N(H)C(O)- =2.1] and the values for the Hydrophobic Group Numbers are [aliphatic/aromatic carbon = 0.475 & aliphatic carbon atoms between polar groups are 1/2 the value of an aliphatic carbon in a hydrocarbon chain = (0.475)/2]. For reference, an H.L.B. value >7 indicates that the material is preferentially water soluble and an H.L.B. value <7 indicates increasing surface-activity and hydrophobicity.

Table A

H.L.B. Values Provided by Various Peroxyacids

| Activator/Preformed Peroxyacid | Abbreviation | Peroxyacid | H.L.B. Corresponding Carboxylic Acid |
|---|---|---|---|
| Tetra Acetyl Ethylene Diamine | TAED | $CH_3C(O)OOH$ | 8.6 |
| DiPeroxyDodecane Dioic Acid | DPDDA | $HOO(O)C(CH_2)_{10}C(O)OOH$ | 6.5 |
| Nonyl Amide of Peroxy Succinic Acid | NAPSA | $CH_3(CH_2)_8N(H)C(O)(CH_2)_2C(O)OOH$ | 6.4 |
| BenzoylOxyBenzene Sulfonate | BOBS | $C_6H_5C(O)OOH$ | 6.3 |
| Nonyl Amide of Peroxy Adipic Acid | NAPAA | $CH_3(CH_2)_8N(H)C(O)(CH_2)_4C(O)OOH$ | 6.0 |
| NonanoylOxyBen-zene Sulfonate | NOBS | $CH_3(CH_2)_7C(O)OOH$ | 5.3 |
| DecanoylOxyBen-zene Sulfonate | DOBS | $CH_3(CH_2)_8C(O)OOH$ | 4.8 |
| PerLauric Acid | PLA | $CH_3(CH_2)_{10}C(O)OOH$ | 3.9 |

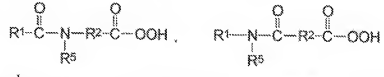
As noted hereinbefore, a preferred range of H.L.B. values (of the corresponding carboxylic acid) for the peroxyacids of the present invention (whether added directly or generated in situ) ranges from about 3.0 to about 6.5. A more preferred range of H.L.B. values (as the carboxylic acid) for the peroxyacids useful in the present invention (whether added directly or generated in situ) range from about 4.0 to 6.5. The most preferred range of H.L.B. values (as the carboxylic acid) for the peroxyacids of the present invention (whether added directly as generated in situ) ranges from about 4.0 to about 6.0.

(a) Peroxyacid

The present invention encompasses detergent compositions comprising an effective amount of the protease enzyme and a bleaching system comprising at least about 0.1%, preferably from about 0.1% to about 50%, by weight, of a substantially insoluble organic peroxyacid. The peroxyacid useful herein preferably comprises from about 0.5 to about 20, more preferably from about 1 to about 10, most preferably from about 2 to about 7, wt.% of the composition.

Preferred organic peroxyacids are selected from the group consisting of 4-nonylamino-4-oxoperoxybutyric acid; 6-(nonyl-amino)-6-oxoperoxycaproic acid; 1,12-diperoxydodecanedioic acid; heptyl sulfonylperpropionic acid; decylsulphonyl perpropionic acid; and heptyl-, octyl-, nonyl-, decyl-sulphonylperbutyric acid; and mixtures thereof.

Of the organic peroxyacids, amidoperoxyacids (amide substituted peroxycarboxylic acids) are preferred. Suitable amidoperoxyacids for use herein are described in U.S. Patents 4,634,551 and 4,686,063, both Burns et al., issued January 6, 1987 and August 11, 1987, respectively, both incorporated herein by reference. Suitable amidoperoxyacids are of the formula:



wherein $R^1$ is an alkyl, aryl, or alkaryl group containing from about 1 to about 14 carbon atoms (preferably $R^1$ is an alkyl group containing from about 6 to about 12 carbon atoms), $R^2$ is an alkylene, arylene or alkarylene group containing from about 1 to about 14 carbon atoms (preferably $R^2$ is an alkylene group containing from about 1 to about 6 carbon atoms), and $R^5$ is H or an alkyl, aryl, or alkaryl group containing from about 1 to about 10 carbon atoms (preferably $R^5$ is H). More preferably, $R^1$ is an alkyl group containing from about 8 to about 10 carbon atoms, and $R^2$ is an alkylene group containing from about 2 to about 4 carbon atoms.

Another preferred preformed peracid includes E-phthalimido-peroxycaproic acid ("PAP"). See for example U.S. Patent Nos. 5,487,818, 5,310,934, 5,246,620, 5,279,757 and 5,132,431.

Other suitable peroxycaproic acids include, but are not limited to, N,N'-terephthaloyl-di-(6-amino-peroxycaproic acid) ("TPCAP") and others described in U.S. Patent No. 5,770,551. Additionally, N-nonanoyl-6-amino peroxycaproic acid ("NAPCA") can also be used as a peracid. See U.S. Patent Nos. 5,523,434, 4,634,551 and 4,852,989.

Also suitable for use herein are peroxyfumarates, which are described in U.S. Patent 4,852,989, Burns et al., issued August 1, 1989, incorporated herein by reference, and sulfone peroxyacids (sulfone peroxycarboxylic acids), which are described in U.S. Patents 4,758,369, 4,824,591, and 5,004,558, all Dryoff et al., issued July 19, 1988, April 25, 1989, and April 2, 1991, respectively, all incorporated herein by reference.

Example I of U.S. Patent 4,686,063 contains one description of the synthesis of NAPSA, from column 8, line 40 to column 9, line 5, and NAPAA, from column 9, line 15 to column 9, line 65. At the end of the amidoperoxyacid synthesis, the reaction is

quenched with water, filtered, washed with water to remove some excess sulfuric acid (or other strong acid with which the peroxyacid was made), and filtered again.

The amidoperoxyacid wet cake thus obtained can be contacted with a phosphate buffer solution at a pH between about 3.5 and 6, preferably between about 4 and 5, according to U.S. Patent 4,909,953, Sadlowski et al., issued March 20, 1990, which is incorporated herein by reference.

Other agents for storage stabilization or exotherm control can be added to the amidoperoxyacid before incorporation into the final product. For example, boric acid, an exotherm control agent disclosed in U.S. Patent 4,686,063, Burns, issued August 11, 1987 and incorporated herein, can be mixed with the amidoperoxyacid (which has been washed in phosphate buffer) in about a 2:1 peracid:boric acid ratio. The phosphate buffer washed amidoperoxyacid can also be mixed with appropriate amounts of dipicolinic acid and tetrasodium pyrophosphate, a chelating stabilization system. Chelants can optionally be included in the phosphate buffer before contact with the wet cake.

The wet cake is preferably made up of particles with an average particle diameter of from about 0.1 to about 260 microns, preferably from about 10 to about 100 microns, and most preferably from about 30 to about 60 microns. Small particle size NAPAA crystals are desired herein. See U.S. Patent 5,055,218, Getty et al., issued October 8, 1991, which is incorporated herein by reference.

NAPAA filter cake herein is preferably washed twice in phosphate buffer. It has been found that two successive phosphate buffer washes lend optimal stability to NAPAA.

Particulate (solid), organic peroxyacids with a theoretical AvO (available oxygen) of between about 3 and about 12, most preferably between 5 and 7, are preferred.

Most preferred for use herein is NAPAA. Another name for the nonylamide of peroxyadipic acid ("NAPAA") is 6-(nonylamino)-6-oxoperoxycaproic acid. The chemical formula for NAPAA is:

$$CH_3(CH_2)_8N(H)-C(=O)-(CH_2)_4COOH$$

The molecular weight of NAPAA is 287.4.

Detergent compositions and bleaching compositions containing NAPAA provide extremely effective and efficient surface bleaching of textiles. Stains and/or soils are removed from the textiles. These compositions are particularly effective at removing dingy soils from textiles.

NAPAA's polar amide or substituted amide moiety results in a peroxyacid which has a very low vapor pressure and thus possesses a low odor profile as well as excellent bleaching performance. It is believed that the polarity of the amide group results in a reduction of vapor pressure of the peroxyacid, and an increase in melting point.

NAPAA can be used directly as a bleaching agent. It has a reduced vapor pressure and a good odor profile in laundry applications.

NAPAA can be prepared by, for example, first reacting NAAA (monononyl amide of adipic acid), sulfuric acid, and hydrogen peroxide. The reaction product is quenched by addition to ice water followed by filtration, washing with distilled water, and final suction filtration to recover the wet cake. Washing can be continued until the pH of the filtrate is neutral.

It is also preferred that the NAPAA pH (10% solids in water) be between about 4.2 and 4.8. Surprisingly, this pH results in more thermally stable particles.

(b) Bleaching Systems - Bleach Activator and Peroxygen Bleaching Compound

(i) Bleach Activators

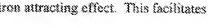
The bleach activator for the bleaching systems useful herein preferably has the following structure:

wherein R is an alkyl group containing from about 5 to about 18 carbon atoms wherein the longest linear alkyl chain extending from and including the carbonyl carbon contains from about 6 to about 10 carbon atoms and L is a leaving group, the conjugate acid of which has a pKa in the range of from about 4 to about 13, preferably from about 6 to about 11, most preferably from about 8 to about 11.

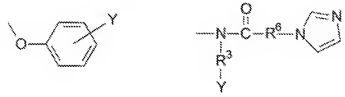
L can be essentially any suitable leaving group. A leaving group is any group that is displaced from the bleach activator as a consequence of the nucleophilic attack on the bleach activator by the perhydroxide anion. This, the perhydrolysis reaction, results in the formation of the percarboxylic acid. Generally, for a group to be a suitable leaving group it must exert an electron attracting effect. This facilitates the nucleophilic attach by the perhydroxide anion.

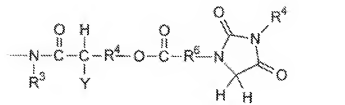
The L group must be sufficiently reactive for the reaction to occur within the optimum time frame (e.g., a wash cycle). However, if L is too reactive, this activator will be difficult to stabilize. These characteristics are generally paralleled by the pKa of the conjugate acid of the leaving group, although exceptions to this convention are known.

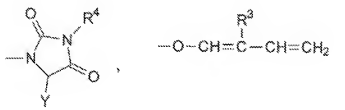
Preferred bleach activators are those of the general formula:

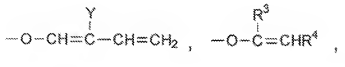


wherein $R^1$ is an alkyl group containing from about 6 to about 12 carbon atoms, $R^2$ is an alkylene containing from 1 to about 6 carbon atoms, $R^5$ is H or alkyl, aryl, or alkaryl containing from about 1 to about 10 carbon atoms, and L is selected from the group consisting of:







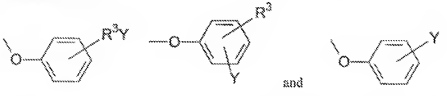


wherein $R^6$ is an alkylene, arylene, or alkarylene group containing from about 1 to about 14 carbon atoms, $R^3$ is an alkyl chain containing from about 1 to about 8 carbon atoms, $R^4$ is H or $R^3$, and Y is H or a solubilizing group. Y is preferably selected from the group consisting of -$SO_3$-M+, -COO-M+, -$SO_4$-M+, (-N+$R'_3$)X- and O←N($R'_3$), wherein R' is an alkyl chain containing from about 1 to about 4 carbon atoms, M is a cation which provides solubility to the bleach activator and X is an anion which provides

solubility to the bleach activator. Preferably, M is an alkali metal, ammonium or substituted ammonium cation, with sodium and potassium being most preferred, and X is an anion selected from the group consisting of halide, hydroxide, methylsulfate and acetate anions. More preferably, Y is $-SO_3$-M+ and -COO-M+. It should be noted that bleach activators with a leaving group that does not contain a solubilizing group should be well dispersed in the bleach solution in order to assist in their dissolution. Preferred is:

wherein $R^3$ is as defined above and Y is $-SO_3$-M+ or -COO-M+ wherein M is as defined above.

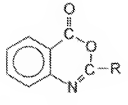
Especially preferred bleach activators are those wherein $R^1$ is a linear alkyl chain containing from about 6 to about 12 carbon atoms, $R^2$ is a linear alkylene chain containing from about 2 to about 6 carbon atoms, $R^5$ is H, and L is selected from the group consisting of:



and

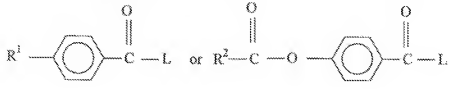
wherein $R^3$ is as defined above, Y is $-SO_3$-M+ or -COO-M+ and M is as defined above.

A preferred bleach activator is:



wherein R is H, alkyl, aryl or alkaryl. This is described in U.S. Patent 4,966,723, Hodge et al., incorporated by reference herein.

Preferred bleach activators are:

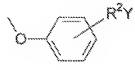
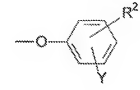
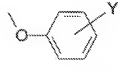


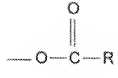
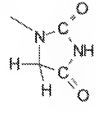
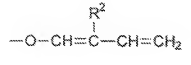
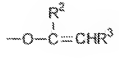
wherein $R^1$ is H or an alkyl group containing from about 1 to about 6 carbon atoms and $R^2$ is an alkyl group containing from about 1 to about 6 carbon atoms and L is as defined above.

Preferred bleach activators are also those of the above general formula wherein L is as defined in the general formula, and $R^1$ is H or an alkyl group containing from about 1 to about 4 carbon atoms.

Even more preferred are bleach activators of the above general formula wherein L is as defined in the general formula and $R^1$ is a H.

More preferred bleach activators are those of the above general formula wherein R is a linear alkyl chain containing from about 5 to about 9 and preferably from about 6 to about 8 carbon atoms and L is selected from the group consisting of:

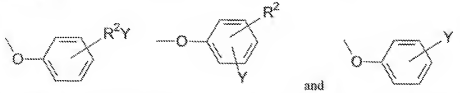




wherein R, $R^2$, $R^3$ and Y are as defined above.

Particularly preferred bleach activators are those of the above general formula wherein R is an alkyl group containing from about 5 to about 12 carbon atoms wherein the longest linear portion of the alkyl chain extending from and including the carbonyl

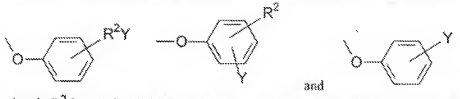
carbon is from about 6 to about 10 carbon atoms, and L is selected from the group consisting of:



and

wherein $R^2$ is an alkyl chain containing from about 1 to about 8 carbon atoms, and Y is -SO-3M+ or -COO-M+ wherein M is an alkali metal, ammonium or substituted ammonium cation.

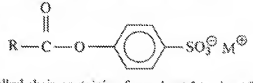
Especially preferred bleach activators are those of the above general formula wherein R is a linear alkyl chain containing from about 5 to about 9 and preferably from about 6 to about 8 carbon atoms and L is selected from the group consisting of:



and

wherein $R^2$ is as defined above and Y is -SO-3M+ or -COO-M+ wherein M is as defined above.

The most preferred bleach activators have the formula:

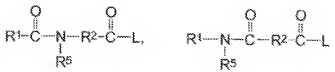


wherein R is a linear alkyl chain containing from about 5 to about 9 and preferably from about 6 to about 8 carbon atoms and M is sodium or potassium.

Preferably, the bleach activator herein is sodium nonanoyloxybenzenesulfonate (NOBS) or sodium benzoyloxybenzenesulfonate (BOBS).

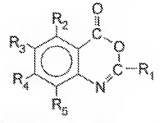
Further particularly preferred for use in the present invention bleaching compositions are the following bleach activators which are particularly safe for use with machines having natural rubber parts. This is believed to be the result of not producing oily diacylperoxide (DAP) species by the perhydrolysis reaction of these amido acid-derived bleach activators, but rather forming insoluble crystalline solid DAP's. These solids are believed to not form a coating film and thus natural rubber parts are not exposed to DAP's for extended periods of time. These preferred bleach activators are members selected from the group consisting of:

a) a bleach activator of the general formula:



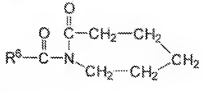
or mixtures thereof, wherein $R^1$ is an alkyl, aryl, or alkaryl group containing from about 1 to about 14 carbon atoms, $R^2$ is an alkylene, arylene or alkarylene group containing from about 1 to about 14 carbon atoms, $R^5$ is H or an alkyl, aryl, or alkaryl group containing from about 1 to about 10 carbon atoms, and L is a leaving group;

b) benzoxazin-type bleach activators of the general formula:



wherein $R_1$ is H, alkyl, alkaryl, aryl, arylalkyl, and wherein $R_2$, $R_3$, $R_4$, and $R_5$ may be the same or different substituents selected from H, halogen, alkyl, alkenyl, aryl, hydroxyl, alkoxyl, amino, alkylamino, $COOR_6$ (wherein $R_6$ is H or an alkyl group) and carbonyl functions;

c) N-acyl caprolactam bleach activators of the formula:



wherein $R^6$ is H or an alkyl, aryl, alkoxyaryl or alkaryl group containing from 1 to 12 carbons; and

d) mixtures of a), b) and c).

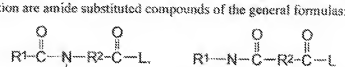
Preferred bleach activators of type a) are those wherein $R^1$ is an alkyl group containing from about 6 to about 12 carbon atoms, $R^2$ contains from about 1 to about 8 carbon atoms, and $R^5$ is H or methyl. Particularly preferred bleach activators are those of the above general formulas wherein $R^1$ is an alkyl group containing from about 7 to about 10 carbon atoms and $R^2$ contains from about 4 to about 5 carbon atoms.

Preferred bleach activators of type b) are those wherein $R_2$, $R_3$, $R_4$, and $R_5$ are H and $R_1$ is a phenyl group.

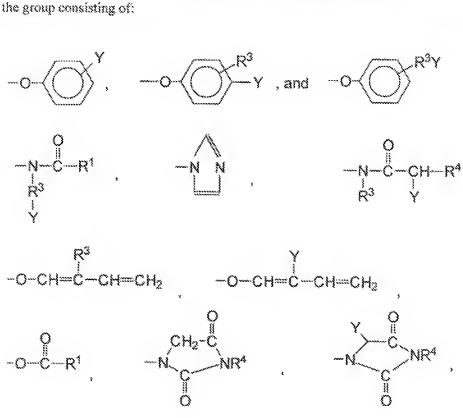
The preferred acyl moieties of said N-acyl caprolactam bleach activators of type c) have the formula $R^6$-CO- wherein $R^6$ is H or an alkyl, aryl, alkoxyaryl, or alkaryl

group containing from 1 to 12 carbons, preferably from 6 to 12 carbon atoms. In highly preferred embodiments, $R^6$ is a member selected from the group consisting of phenyl, heptyl, octyl, nonyl, 2,4,4-trimethylpentyl, decenyl and mixtures thereof.

- Amido Derived Bleach Activators - The bleach activators of type a) employed in the present invention are amide substituted compounds of the general formulas:



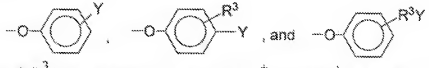
or mixtures thereof, wherein $R^1$, $R^2$ and $R^5$ are as defined above and L can be essentially any suitable leaving group. Preferred bleach activators are those of the above general formula wherein $R^1$, $R^2$ and $R^5$ are as defined for the peroxyacid and L is selected from the group consisting of:



and mixtures thereof, wherein $R^1$ is an alkyl, aryl, or alkaryl group containing from about 1 to about 14 carbon atoms, $R^3$ is an alkyl chain containing from 1 to about 8 carbon atoms, $R^4$ is H or $R^3$, and Y is H or a solubilizing group.

The preferred solubilizing groups are $-SO_3^-M^+$, $-CO_2^-M^+$, $-SO_4^-M^+$, $-N^+(R^3)_4X^-$ and $O<N(R^3)_3$ and most preferably $-SO_3^-M^+$ and $-CO_2^-M^+$ wherein $R^3$ is an alkyl chain containing from about 1 to about 4 carbon atoms, M is a cation which provides solubility to the bleach activator and X is an anion which provides solubility to the bleach activator. Preferably, M is an alkali metal, ammonium or substituted ammonium cation, with sodium and potassium being most preferred, and X is a halide, hydroxide, methylsulfate or acetate anion. It should be noted that bleach activators with a leaving group that does not contain a solubilizing groups should be well dispersed in the bleaching solution in order to assist in their dissolution.

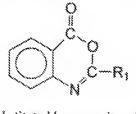
Preferred bleach activators are those of the above general formula wherein L is selected from the group consisting of:



wherein $R^3$ is as defined above and Y is $-SO_3^-M^+$ or $-CO_2^-M^+$ wherein M is as defined above.

Another important class of bleach activators, including those of type b) and type c), provide organic peracids as described herein by ring-opening as a consequence of the nucleophilic attack on the carbonyl carbon of the cyclic ring by the perhydroxide anion. For instance, this ring-opening reaction in type c) activators involves attack at the caprolactam ring carbonyl by hydrogen peroxide or its anion. Since attack of an acyl caprolactam by hydrogen peroxide or its anion occurs preferably at the exocyclic carbonyl, obtaining a significant fraction of ring-opening may require a catalyst. Another example of ring-opening bleach activators can be found in type b) activators, such as those disclosed in U.S. Patent 4,966,723, Hodge et al, issued Oct. 30, 1990.

- Benzoxazin-type Bleach Activators - Such activator compounds disclosed by Hodge include the activators of the benzoxazin-type, having the formula:



including the substituted benzoxazins of the type